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JOURNAL OF GENETICS

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CORRIGENDA IN VOL. VII.

P. 201, line 13, for $K_3 = \frac{(1+\sqrt{5})}{5} s_0 + \frac{(1-\sqrt{5})}{5} (s_0 - 4r_0 t_0)$

read $K_3 = \frac{(1+\sqrt{5})}{5} s_0 + \frac{(1-\sqrt{5})}{5} (s_0^2 - 4r_0 t_0)$.

P. 201, last line, for homozygotes read heterozygotes.

CORRIGENDA IN VOL. VIII.

P. 74, line 27, for equally 3 : 1 read equally 1 : 1.

P. 79, last line but one, for (17 - 1) read (17 - 13).

Pl. II, fig. 5. The stem shews some brown pigment in the original drawing which has been lost in the reproduction.

GENETIC STUDIES IN RABBITS.

I. ON THE INHERITANCE OF WEIGHT.

By R. C. PUNNETT, F.R.S.,
AND THE LATE MAJOR P. G. BAILEY, R.F.A.

(With Twelve text-figures.)

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INTRODUCTION.

SEVERAL investigators have published data relating to the inheritance of weight in rabbits. The earliest experiments appear to be those of Huth ('87) which were undertaken with the idea of ascertaining whether continuous inbreeding through brother and sister mating led to any diminution of size. The results are not published in a form which allows of analysis, since the individual weights of the different animals, whether parents or progeny, are not given. Huth however came to the conclusion that inbreeding over six generations did not lead to any deterioration in size. More recently Castle ('09) took up the question of size inheritance in this species. He admits that his statistics are unsatisfactory as he was unable to keep many of his animals until the adult state was reached. Most of his comparisons were consequently

made at an age of 18 weeks, and this, as will appear later, is too early for trustworthy conclusions. Nor did he realize the marked sexual differences which may occur, and which must, as we shall explain later, be taken into account in work of this nature. He came to the conclusion that weight inheritance is blending in character, and that neither dominance nor segregation in the Mendelian sense is recognisable.

Castle's work was done before Nilsson-Ehle had put forward the well known hypothesis of multiple factors. More recently MacDowell ('14), in continuing Castle's experiments, has come to the conclusion that his data are all in accordance with this hypothesis. MacDowell's work is mainly concerned with skeletal measurements, and though periodical weighings of his animals were made he does not attach much significance to the results, pointing out various factors which tend to militate against accuracy.

Quite recently a few observations on the growth of rabbits have been published by Davies ('17) who states that when a small breed is crossed with a medium sized one the young approximate to the size of the smaller parent. But he admits that the cross was only made in one way using the animal of the smaller breed as the mother. He is inclined to consider that the reciprocal cross may give a different result, as he holds that larger size depends more upon the mother than upon the father.

We cannot yet pretend to know very much about the inheritance of weight in the rabbit. It is clear however that investigations of this nature in the mammal do not yield such clean-cut results as in birds (cf. Phillips ('12), Punnett and Bailey ('14)), and it is evident that a great deal of laborious work must yet be done before we can understand a process of which the theoretical, as well as the economic, importance is so considerable. We do not therefore propose to discuss the matter from a general point of view until we are in possession of fuller and more complete data than those of which we may now proceed to give some account.

General Account. Our experiments were started in 1912. The breeds originally chosen were the Flemish¹, one of the largest of rabbits, and a strain of mixed Himalayan-Dutch-Havana origin which had been formed in the course of some earlier experiments on coat colour. We shall refer to this as the Flemish-mixed cross. The three breeds which entered into this strain are all on the small side and on the whole differ

¹ We are indebted to the kindness of Dr R. N. Salaman for procuring these animals for us.

little from one another in point of size¹. The strain was chosen on account of its peculiar pattern, since it was hoped, by crossing it with a self-coloured race, to obtain data on the inheritance of white markings on the coat. These experiments are still in progress and the results will be presented in some future paper. In 1915 a further set of experiments was started, using as parent races the Flemish and the Polish², the latter being the smallest breed of rabbits in domestication.

The work, so far as it has gone, is necessarily of a preliminary nature. We did not begin by crossing strains of uniform size, a point of the first importance in work of this kind. Our reason for not doing so was of course the impossibility of finding them. The "pure" breeds of rabbits of which we have had experience shew fluctuations of size, often considerable, which cannot be put down to ill health, alteration of conditions, and so forth; and we have little doubt that this is true for all recognised breeds. The student of genetics, like the chemist, often has to purify his raw material as a preliminary to critical research. In the present case this is a matter of some years. But while the standardisation of our material was proceeding we carried on the crossing experiments referred to above with a view to obtaining useful experience for more extensive and critical work in the future. That the results hitherto obtained are indecisive is no disappointment, having regard to the nature of the material and to the resources at our command. But in spite of the limitations of our data we believe that they will prove of service in directing the attention of other workers along these lines to points hitherto unconsidered in connection with the inheritance of weight. On some of these it is hoped that further light will be thrown when the material now being worked up becomes available for crossing purposes.

A. Flemish-mixed cross. The original Flemish ♂ (N 169) was mated with two does of the Himalayan-Dutch-Havana strain, N 17 and N 19, which weighed respectively 6 lbs. 2 oz. and 6 lbs. 6 oz. These two does were closely related to one another as is shewn by the accompanying pedigree (Fig. 1). To what extent the members of this strain of mixed origin varied in weight we are unable to say, since records bearing upon

¹ Davies ('17) speaks of modern Polish, Himalayan, Dutch, and Tan varieties approximating closely in size to their wild prototype, and he also mentions 3 lbs. as the weight of a Himalayan doe. This is very much less than the weight of such a doe 10—15 years back. Apparently the trend of the fancy has been towards smaller size for these breeds in recent years.

² For these animals and for sundry information about them our thanks are due to Professor J. Stanley Gardiner.

this point were not kept while it was being formed¹. The experiments involving ♀ N 19 are less extensive and may be considered first. Six F_1 animals were reared (cf. Table II, p. 22) and, as shewn graphically in Fig. 2², they were intermediate in size with the exception of one indi-

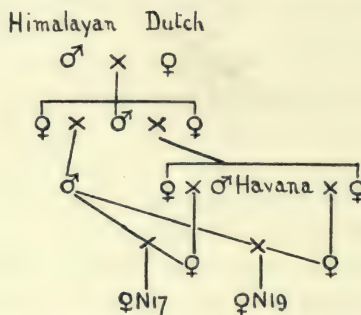


Fig. 1. Pedigree of the two does, ♀ N 17 and ♀ N 19, used in the Flemish-mixed cross.

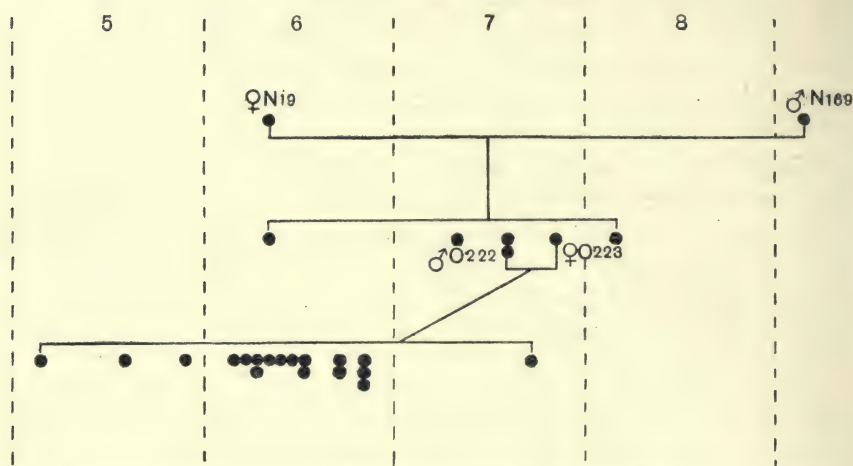


Fig. 2. Graphic representation of weight distribution in F_1 and F_2 generations from the Flemish-mixed cross, ♀ N 19 \times ♂ N 169. The numbers above each of the columns separated by broken lines denote lbs. Each black circle represents an individual and is placed according to the weight of the individual in lbs. and oz. The record number of those rabbits which have been mated together is given, cf. Tables I and II, pp. 21—22.

¹ Considerable numbers of animals involving these three breeds were reared by me between 1907 and 1912. I do not recall any marked differences in size. The great majority, if not all of them, were probably between 5 and 7½ lbs. R. C. P.

² Unless any statement to the contrary is made in Tables I—V each weight given is the maximum attained during the first twelve months (cf. p. 7).

vidual (σ O 224) which was of the same weight as its mother. An F_2 generation (cf. Table III, p. 23) was reared from two members of the F_1 family which were nearly of the same size. It consisted of 18 individuals and exhibited the considerable range of variation shewn in Fig. 2. Points of interest are, (a) that the average weight is close to that of the original small parent (N 19) and considerably less than that of the F_1 generation, (b) that only one animal attained the mean parental weight, and (c) that in several cases animals were produced which were much smaller than the original small parent (N 19). As compared with F_1 the F_2 generation shews a marked shifting towards smaller size. The numbers are too small to draw any deduction of value as to there being an increase of variability in F_2 as compared with F_1 .

The experiments with ♀ N 17 are more extensive and have been carried through several generations. The 15 F_1 animals reared shew a wide range of variation, some of them being nearly as small as their mother while others are considerably heavier than their father (cf. Table II, p. 22, and Fig. 3). Four F_1 animals involving three different matings were subsequently used to give an F_2 generation. These four animals were all within 1 lb. of one another. The F_2 generation taken together exhibits great variability (cf. Fig. 3) extending some way beyond the range of the original parents on either side. Though the mean of the F_2 generation as compared with that of the F_1 generation is shifted towards the smaller size this is not so marked a phenomenon as in the preceding case. In this connection we shall have more to say later.

It was our original intention to breed an F_3 generation from a pair of the heaviest F_2 animals as well as from a pair of the lightest. Consideration of space however compelled us to restrict ourselves to the latter part of the programme. An F_3 generation of 23 was reared from two of the smaller F_2 animals (♀ O 187 and σ O 192). As compared with their parents the mean of this generation again shews some shifting to the left in Fig. 3. At this point it was decided to try the effects of continuous inbreeding from the smallest of the F_3 generation in order to ascertain whether a fairly true-breeding strain of small size could eventually be extracted. It is hoped to expand this part of the experiment in the near future and to test the effects of close inbreeding with considerably larger numbers.

B. Flemish-Polish cross. A small Polish buck weighing 3 lbs. was successfully mated with two Flemish does. In one case the doe (O 138) was more than three times as heavy as the Polish buck. A single pair of F_1 animals was reared and proved to be nearly intermediate in size

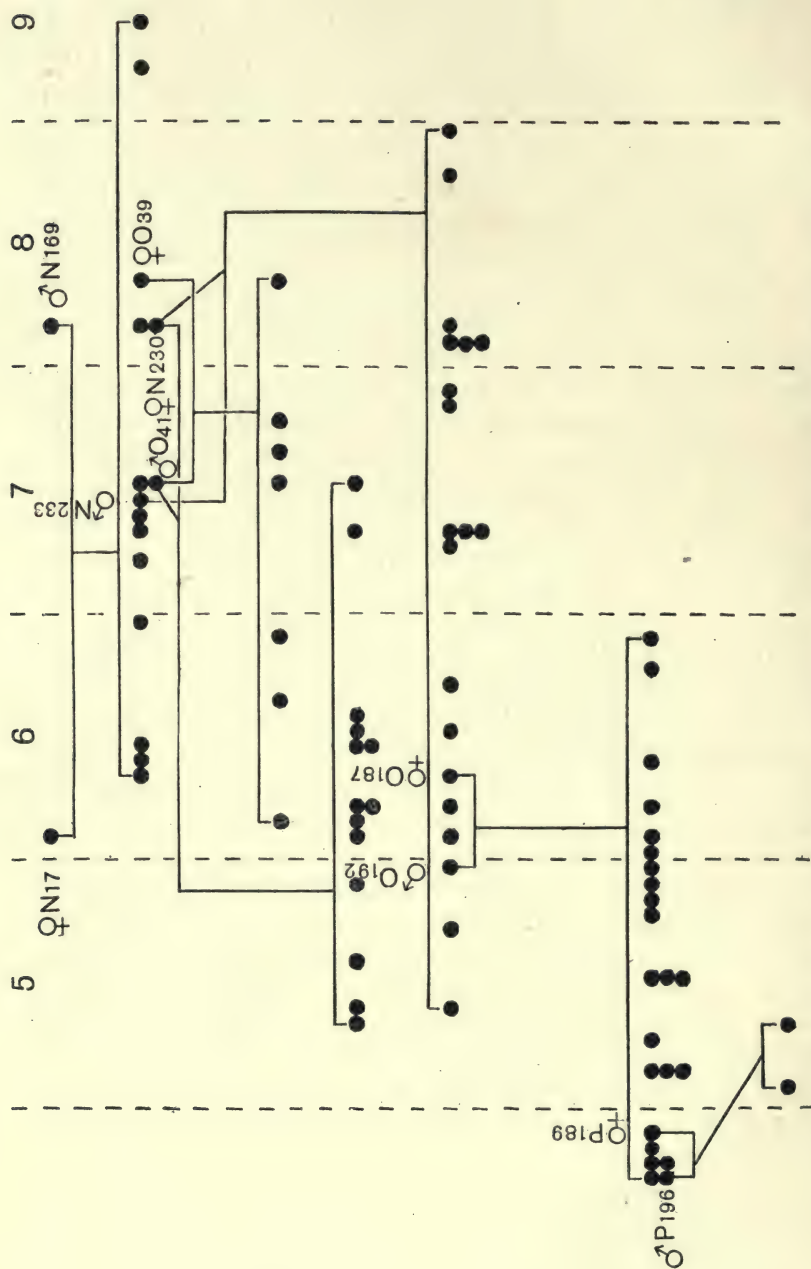


Fig. 3. Graphic representation of weight distribution in F_1 — F_4 generations from the Flemish mixed cross, ♀N17 × ♂N169. For further details as to exact weights, see Tables I—IV, pp. 21—24.

between the parents (cf. Table V, p. 25, and Fig. 4). Two litters were bred from this pair giving 11 F_2 animals in all. The average size of these was distinctly less than that of the parents, but, although the variability was considerable, nothing was produced approaching the small size of the grandfather. Possibly this may be due merely to the small number of F_2 animals reared.

From the other Flemish doe (O 203) the numbers are greater. The four F_1 animals were closely intermediate in size between the parents (cf. Table V, p. 25, and Fig. 5). From these two F_1 pairs 36 F_2 offspring were reared to maturity. The results are closely comparable with those obtained from the other Flemish-Polish mating. There is considerable variability in F_2 though very few individuals reached the F_1 size and only one exceeded it, and that but slightly. No individual shows any approach to the large size of the Flemish. Some shewed a fairly close approach to the small size of the Polish grandfather, and it seems not unlikely that with larger numbers animals of this extreme small size might have reappeared in F_2 .

Taking all of the experiments together the chief point of interest about them is, unquestionably, the failure of the larger form to reappear in F_2 in certain of the crosses. It is marked in the Flemish-Polish crosses and evident in the F_2 family from N 19. On the other hand it does not seem to occur in the other F_2 generation from the Flemish-mixed cross, viz. that from N 17. The non-appearance of an expected class in F_2 has occasionally been recorded for sundry characters, but the only instance which we can recall in connection with size is that of East's *Nicotiana* crosses ('17), where, from the cross between *N. Langsdorffii* and *N. alata*, the small corolla length was recovered in F_2 without difficulty while nothing approaching the long corolla of *N. alata* reappeared. With this brief account of the crossing experiments we may now proceed to discuss various points to which they have given rise.

The Growth Curve. We have already stated that the weight recorded in our tables is the maximum weight attained by the animal during the first twelve months of life¹. Generally speaking, a rabbit grows rapidly during the first half year of its existence, after which the monthly increment gradually becomes smaller. Somewhere between 7 and 10 months in the smaller breeds a maximum weight is reached, after which there is usually some decline, connected probably with the fulfilment of sexual maturity. Later on an increase in weight is again

¹ With some few exceptions where growth was slow and maturity delayed. These are noted in Tables I—V, pp. 21—25.

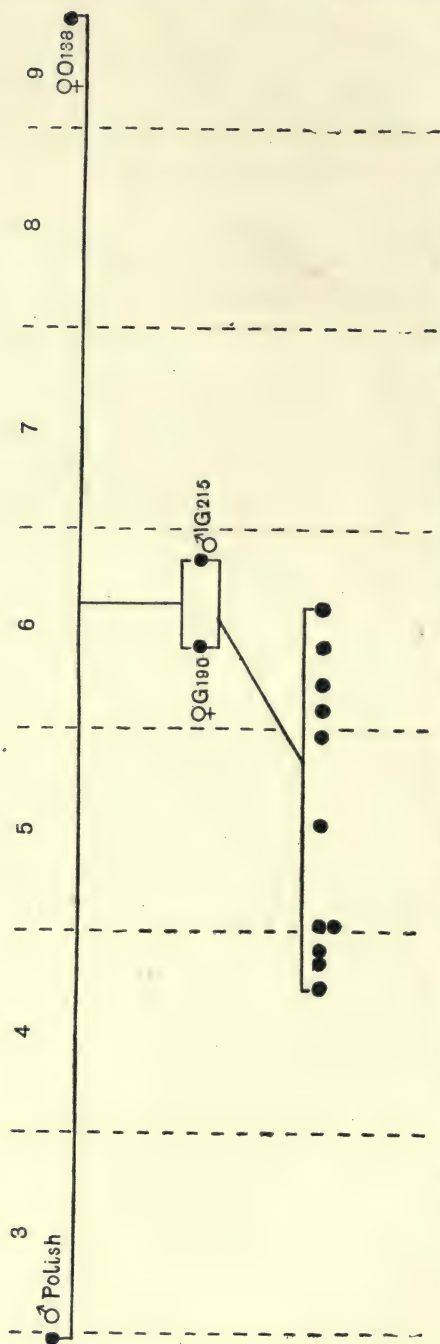


Fig. 4. Graphic representation of weight distribution in F_1 and F_2 generations from Flemish-Polish cross, $\varnothing O138 \times \sigma$ Polish. For further details as to exact weights, see Table V, p. 25.

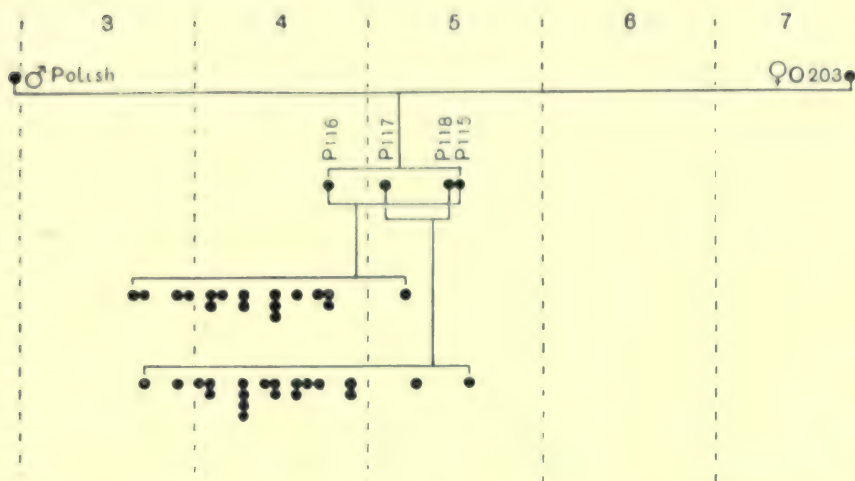


Fig. 5. Graphic representation of weight distribution in F_1 and F_2 generations from Flemish-Polish cross, ♀ O 203 × ♂ Polish. For further details as to exact weights, see Table V, p. 25.

noticeable in most cases, and frequently the weight eventually attained is greater than the maximum registered before the age of 12 months. This later increase is more marked in does than in bucks, and is probably connected with the deposition of fat. The general features of the growth curve are illustrated in Fig. 6, which shows graphically the rate

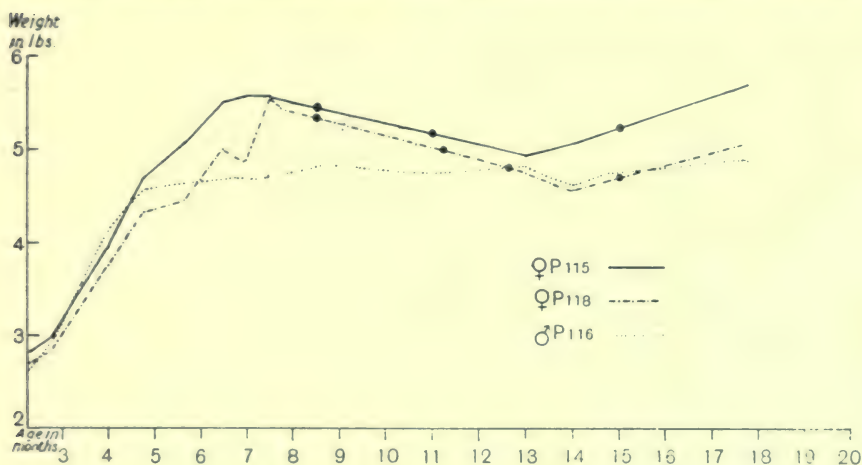


Fig. 6. Growth curves of 3 F_1 animals from Flemish-Polish cross. The curve for ♂ P 117 is almost identical with that for ♂ P 116 and has been omitted for clearness. The black dots on the two female curves indicate the ages at which litters were produced.

of growth of three F_1 animals from the Flemish-Polish cross (cf. Table V, p. 25). In the large breed to which our experience has been limited, the Flemish, growth is relatively slower and the curve is consequently more drawn out. In the case of the four animals whose growth is shewn on Fig. 7 the maximum weight, prior to cessation of growth, was not attained until an age of 14–15 months was reached, and it was also noticed that these animals were very slow in arriving at sexual maturity. Nevertheless the usual decline in weight subsequently set in, and in this respect the heavier breed conforms to the type of the curve exhibited by the lighter animals of Fig. 6. It would be of interest to learn whether a rise in weight occurs later in life, but owing to lack of space we have been unable to keep the animals long enough to test this point. It will be noticed from Figs. 6 and 7 that at seven months the F_1 animals

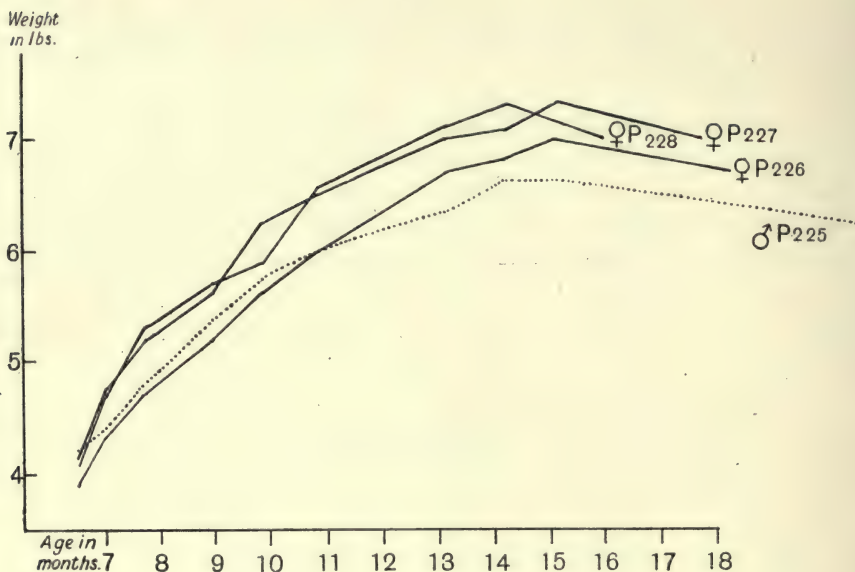


Fig. 7. Growth curves for 4 Flemish rabbits. (Cf. Table I, p. 21.)

were markedly heavier than the Flemish. Their early growth was much more rapid and their sexual maturity much earlier. The Flemish animals which provided the data for Fig. 7 had been inbred for several generations (cf. Fig. 12, p. 20), and it is not impossible that inbreeding may be connected with slow growth and delayed sexual maturity. Early maturity is not necessarily connected with small size as is shewn

graphically by the record of three Polish rabbits in Fig. 8. The ♀ (Q82) was over 10 months old before any decline of weight set in, while neither of the ♂♂ attained their full weight until 11 and 12 months respectively. Here again sexual maturity was late. Though the doe was run with the buck several times earlier it was not until she was 11 months old that she produced a litter. It is worthy of note that these animals are the product of certainly two generations of brother × sister mating. Whether the inbreeding extended further back we are unable to say.

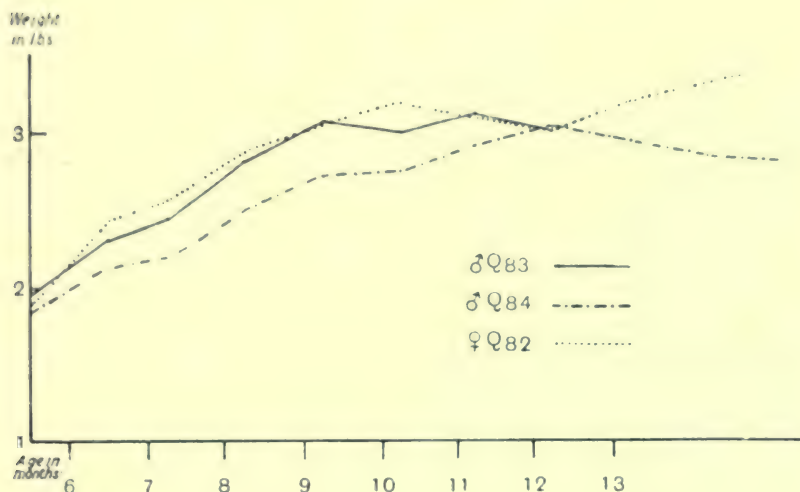


Fig. 8. Growth curves for 3 Polish rabbits.

A further point of interest concerns the growth of the F_2 animals from one of the Flemish-Polish crosses, viz. that from the Flemish ♀ O203. Growth curves were made for all of these animals and it was found that the period in which maximum growth was reached before the temporary decline set in varied from 8–13 months. The distribution of these animals over the different periods is shewn in Fig. 9. In no case was maturity reached as early as in the F_1 parents, while in several cases it was delayed until the age of 13 months (cf. Fig. 10). As might have been expected the smaller F_2 animals mature on the whole earlier than the larger ones. The average weight for those maturing at 8 months is 4 lbs. 2½ oz. and this increases fairly regularly, having regard to the paucity of numbers, up to the average weight of 5 lbs. 1½ oz. for those maturing at 13 months. Nevertheless a consideration of average weights does not in this case tell the whole story. Fairly large F_2 animals may

mature early, e.g. Q 75 which reached 4 lbs. 8 oz. in 8 months; while on the other hand a small rabbit may mature late, e.g. Q 13 which took 12 months to reach the weight of 3 lbs. 12 oz.

That early maturity is to some extent independent of size is an inference borne out by the results of the Flemish-mixed cross. The distribution of the ages of maturity in relation to weight for three

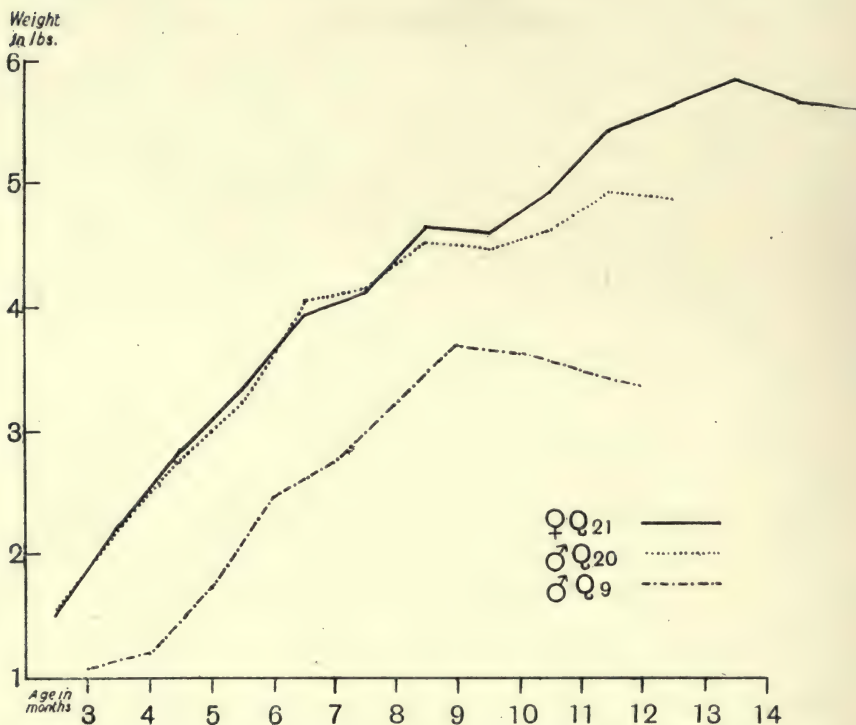


Fig. 9. Growth curves for 3 F_2 animals from Flemish-Polish cross. (Cf. Table V, p. 25.)

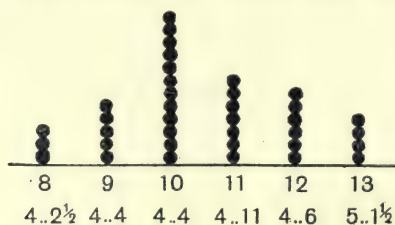


Fig. 10. Shewing distribution of weight in relation to age of maturity in F_2 animals from Flemish-Polish cross. (Cf. Table V, p. 25.)

generations is shown in Fig. 11. No records were kept of the maturity age of the mixed strain which was used. *N* 17 and *N* 19 had their first litters at 11 and 10 months respectively, while other does in the same strain first littered at 9–11 months. The average maturity age of the strain was probably 2–3 months earlier than that of the Flemish. The F_1 animals are notably later in maturing than those from the Flemish-Polish cross, though three of them were distinctly in advance of the rest.

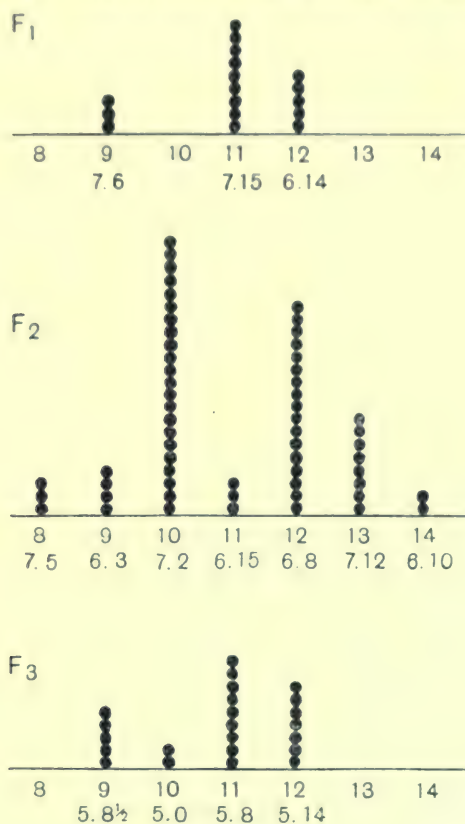


Fig. 11. Shewing distribution of weight in relation to age of maturity in F_1 , F_2 , and F_3 animals of Flemish-mixed cross. (Cf. Tables II–IV, pp. 22–24.)

The F_2 generation, which was raised from among the later maturing of the F_1 animals, shews considerable scatter, maturity in some cases being attained as early as 8 months, while in others it is fully as late as in the Flemish (cf. Fig. 11). The curve appears to be a bimodal one, but the small numbers and the necessarily rough method of classification

hardly warrant us in attaching much significance to this feature. Greater interest attaches to the fact that the maturity age here is less dependent upon weight than in the case of the Flemish-Polish cross. It is true that the group of lowest average weight (6 lbs. 3 oz.) matured early and that the heaviest group (7 lbs. 12 oz.) matured late, but apart from these groups there is no tendency in earlier maturity being associated with lower weight. An association of increased weight with later maturity is rather more marked in the case of the F_3 generation, but the fewer numbers together with the smaller range of variation both detract from its significance.

The possibility that weight might be affected by such features as size of litter, age of doe, and season of birth naturally occurred to us, but examination of our records from this point of view has not led to any positive result. In this respect we agree with MacDowell ('14, pp. 27-28) and we do not think it necessary to go more fully into an analysis of our data in this connection. Castle ('09, p. 39) considers that variation in size is to some extent dependent upon variation in the quality of food. Davies ('17) on the other hand holds that, provided the food supply is adequate, size is a matter of heredity. Our general impression is that the last-named author is right, but we recognise that the point can only be definitely settled by definite experiment. We have endeavoured to keep our animals as far as possible under uniform conditions throughout the year. The hutches are all in a large shed free from draughts and warmed in very cold weather. Until quite recently the animals have always had a liberal allowance of oats together with such green food, roots, etc. as the season affords. They are bedded on sawdust and hay and we have hitherto been very free from sickness—a feature which is at once brought out by the growth curves (cf. McDowell, '14, p. 43).

On the whole our experience suggests the following tentative conclusions with regard to rate of growth and maturity.

(1) The growth curve normally shews a steady rise, gradually flattening with age. About the period of sexual maturity it usually receives a definite check. As a rule this is temporary and the animal subsequently becomes somewhat heavier than it was at maturity¹.

(2) Though animals belonging to large breeds may mature more slowly than those belonging to small breeds it does not follow that age

¹ In the rat growth is apparently continuous, and there would appear to be no definite check at sexual maturity (cf. Donaldson, '15, pp. 66 and 67).

of maturity is closely correlated with size. The very small Polish rabbit matures slowly—probably a good deal more slowly than a larger form such as the Dutch.

(3) Size and early maturity are to some extent transmitted independently. Early maturing large animals as well as late maturing small ones may occur together in the same F_2 family after a cross.

(4) Crossing distinct breeds may in some cases lead to very early maturity in F_1 . In other cases this does not appear to be true.

The data taken together suggest that early maturity may depend upon some factor, or factors, independent of size, though probably a larger rabbit would mature later than a smaller one where both were similarly constituted with regard to the specific genetic factors upon which onset of maturity depended. The present data are far too slender for positive inference, and we do not pretend that they offer more than an indication of a profitable line of enquiry for the future.

SEXUAL DIFFERENCES.

A. *Mature Weight.* It is well known that in many animals the male is normally the heavier of the two sexes when mature. This is probably true of the majority of mammals, and there is experimental evidence that it is also true for certain birds¹. It is not, however, true for rabbits. Our data shew that in some cases the average weight of bucks and does from a given pair of rabbits is approximately equal: in other cases the average weight of the doe is markedly greater than that of the buck. In no case which has come under our notice does the buck certainly exhibit the marked preponderance which may occur in the doe. The position may be indicated by the following abstract (Table A) of the data contained in Tables I–V.

The figures shew that on the whole there is no marked difference between the sexes in the Flemish. Indeed they shew an actual equality for the whole of the animals recorded. This equality is however to be discounted by the fact that a larger proportion of males than of females belongs to the earlier generation when the weights for both sexes were considerably higher (cf. Table I, p. 21). For the later generations considered apart the average weight of the females is rather higher than that of the males. We are inclined to the view that our original Flemish were of mixed constitution with regard to weight factors (whatever these may be) and that the preponderance of weight among the

¹ Cf. Darwin ('91) ii. p. 281; Donaldson ('15); Phillips ('12); Punnett and Bailey ('14).

TABLE A.

	Males		Females	
	Number	Average weight	Number	Average weight
Flemish	13	8 lbs. 2 oz.	12	8 lbs. 2 oz.
„ (later generation) ...	5	7 lbs. 5 oz.	8	7 lbs. 9½ oz.
Ex <i>N</i> 17 × <i>N</i> 169	11	7 lbs. 3 oz.	4	8 lbs. 13 oz.
Ex <i>N</i> 19 × <i>N</i> 169	5	7 lbs. 7 oz.	1	7 lbs. 14 oz.
Above <i>F</i> ₁ generations together...	16	7 lbs. 4 oz.	5	8 lbs. 10 oz.
Ex <i>O</i> 39 × <i>O</i> 41	4	7 lbs. 5 oz.	3	7 lbs. 5 oz.
Ex <i>N</i> 230 × <i>N</i> 233	8	6 lbs. 6 oz.	12	7 lbs. 13 oz.
Ex <i>N</i> 230 × <i>O</i> 41... ..	9	6 lbs. 7 oz.	5	6 lbs. 1 oz.
Ex <i>O</i> 223 × <i>O</i> 222	6	6 lbs. 7 oz.	12	6 lbs. 7 oz.
Ex <i>O</i> 187 × <i>O</i> 192	14	5 lbs. 5 oz.	9	6 lbs. 0 oz.
<i>F</i> ₂ Flemish-Polish (ex <i>O</i> 203) ...	17	4 lbs. 6 oz.	19	4 lbs. 8½ oz.
„ „ (ex <i>O</i> 138)	5	5 lbs. 4 oz.	6	5 lbs. 14 oz.

males in the earlier generation is accidental. In later generations, though the numbers are small, the weights generally shew less fluctuation and the females are consistently heavier¹.

When we come to the Flemish-mixed *F*₁ generation the figures are striking. The does average nearly 1½ lbs. more than the bucks. In view of the small number of the former it is not unlikely that this difference would have been diminished had larger numbers been bred. Nevertheless it is too substantial not to be regarded as having some significance. The *F*₂ generation is of much interest. In two out of the four matings, viz. *O* 39 × *O* 41 and *O* 223 × *O* 222, the sexes average the same weight, in one (*N* 230 × *O* 41) the bucks average rather more than the does, while in the remaining one (*N* 230 × *N* 233) the average weight of the does is very markedly greater than that of the bucks. The only *F*₃ mating was from two animals, ♀ *O* 187 and ♂ *O* 192, both derived from the *F*₂ generation ex *N* 230 × *N* 233. 23 *F*₄ animals were raised, and here again the average weight of the does was markedly greater than that of the bucks.

In the case of the Flemish-Polish crosses the same phenomenon of greater average weight among the does was noticed in the *F*₂ generation ex ♀ *O* 138 × ♂ Polish, while in that from ♀ *O* 203 × Polish the average weight of the two sexes was nearly equal. It is possibly of significance that in the former cross the Flemish doe (*O* 138) was a large one, and in the latter a small one (*O* 203).

Evidently we must recognise that in the rabbit a factor, or factors, are to be found which bring about an increase of size in the doe as

¹ This is supported by the results from a further generation of three Flemish now being reared. At the age of 10 months the ♂ weighed 6 lbs. 0 oz., while the two does were respectively 6 lbs. 8 oz. and 6 lbs. 4 oz.

compared with the buck. That this factor (or factors) may be transmitted by the buck appears probable from the results obtained from ♀ *N* 230. With two brothers of almost exactly the same weight, *N* 233 and *O* 41, she gave in one case a family in which the preponderance of weight was rather on the side of the bucks and in the other case a family in which the does were markedly heavier. That the factor (or factors) may also be transmitted by the doe is indicated by the results of the Flemish-Polish crosses. With the same Polish ♂ the heavier Flemish doe (*O* 138) gave rise to an F_2 generation where the marked discrepancy in weight between the sexes appeared, while with a lighter Flemish ♀ (*O* 203) the F_2 generation shewed little difference in the average weight of the bucks and does. So far as they go, the data suggest the operation of a factor which leads to an increase in the weight of the doe that contains it, though not at all, or only to a slight extent, in that of the buck. Much more work however must be done before we can arrive at certainty on this point, but there seems little doubt but that it is a complication which must be borne in mind in work dealing with the inheritance of weight in this species, and possibly of others also.

B. Rate of Growth. A marked difference in the rate of growth between the two sexes has long been recognised in man where statistics bearing upon the point are more plentiful than in other animals. Up to about 13 years of age the female grows rather more rapidly than the male. After this point the male overtakes the female and eventually becomes markedly heavier¹. This differential growth is probably connected with the earlier onset of puberty in the female sex. Our records allow of some examination of this point in the rabbit, and we have prepared the following brief abstract (Table B) from the data given in Tables II-V

TABLE B.

	Males				Females			
	Number	Average weight ²		Proportion of later growth to 4 months growth	Number	Average weight		Proportion of later growth to 4 months growth
		4 months	12 months			4 months	12 months	
F_1 ex <i>N</i> 17 × <i>N</i> 169	11	4·2½	7·3	·73	4	4·5	8·13	1·04
F_2 ex <i>N</i> 230 × <i>N</i> 233	8	3·9½	6·6	·77	12	3·12	7·13	1·08
F_2 ex <i>N</i> 230 × <i>O</i> 41	7	4·3	6·7½	·54	5	3·8½	6·1½	·72
F_2 ex <i>O</i> 223 × <i>O</i> 222	6	4·5	6·7	·49	12	3·15½	6·8	·63
F_3 ex <i>O</i> 187 × <i>O</i> 192	8	3·11½	5·4½	·42	4	4·0½	6·2	·53
F_2 Polish-Flemish	14	2·3½	4·6	·99	20	2·3	4·8	1·06

¹ According to Darwin (*Descent of Man*, 1891, p. 283) a similar differential sex growth is found in the Scotch deer-hound.

² Under this heading the figure in front of the decimal point denotes pounds while the figure or figures after it denote ounces—e.g. 3·12 is to be read as 3 lbs. 12 oz.

(pp. 22—25). The average weights at 4 months and at 12 months¹ are given separately for the two sexes from matings in which 12 or more offspring were reared. It is evident that up to the age of 4 months there is little difference between the sexes. Where the sexes eventually attain approximately the same weight (e.g. ex *O* 223 × *O* 222), or where the male is slightly heavier (e.g. ex *N* 230 × *O* 41), the weight of the female at 4 months is below that of the male. Where, as in most of the other cases, the female eventually reaches a markedly higher weight she is rather heavier at 4 months. But in all cases the growth subsequent to 4 months is greater in the female than in the male. This is brought out in the last column which shows the proportion of the weight at 4 months that is added between 4 and 12 months. In every mating this proportion is higher in the case of the does.

A point of interest brought out in Table B is that the greater the weight to which the animals eventually attain the greater appears to be the proportional increment added after 4 months, for there is not much difference at 4 months between the larger and the smaller animals in the Flemish-mixed series of experiments. The data, so far as they go, suggest that it might be more economical to breed a larger number of smaller rabbits for killing at about 4 months than to rear a smaller number of larger ones. The latter would have to be kept more than twice as long to gain the advantage of their greater size, and even then the total weight would be less than double that of an equal number of smaller ones killed at 4 months.

The results of the Flemish-Polish cross offer an interesting contrast to those of the Flemish-mixed cross. Though the F_2 animals are all on the small side they are on the whole late in maturing (cf. p. 11). There is practically no difference between the sexes either at 4 months or at maturity which occurs at various ages between 8 and 12 months. This applies only to the F_2 animals from ♀ *O* 203. Full records of the smaller F_2 generation ex ♀ *O* 138 are not available.

One further point of difference in growth between the sexes is brought out by an examination of the F_2 records. It concerns the extent to which the heavier and lighter animals in such a mixture can be distinguished at a relatively early age; or, to put it in another way, how far the animals heavier at maturity give evidence of this at the age

¹ The animals were generally weighed at intervals of a month. Since the litters were produced irregularly the weight at 4 months sometimes refers to animals a little over and sometimes a little under this age. Members of the same litter were of course always weighed at the same age. Weight at 12 months means maximum attained by 12 months.

of 4 months. For testing this point the available records consist of 25 ♂♂ and 36 ♀♀ belonging to the F_2 generation of the Flemish-mixed cross, together with 14 ♂♂ and 20 ♀♀ of the F_2 generation from the Flemish-Polish cross. Both ♂♂ and ♀♀ in each case have

TABLE C.

	Average weight of males under 6 lbs. 8 oz.		Average weight of males of 6 lbs. 8 oz. and over		Average weight of females under 7 lbs.		Average weight of females over 7 lbs.	
	At 4 months	At 12 months	At 4 months	At 12 months	At 4 months	At 12 months	At 4 months	At 12 months
F_2 generation from Flemish-mixed cross	3.5	6.1	4.5	7.2	3.13½	6.2	3.13	7.15½
	Average weight of males under 4 lbs. 8 oz.		Average weight of males of 4 lbs. 8 oz. and over		Average weight of females under 4 lbs. 10 oz.		Average weight of females of 4 lbs. 10 oz. and over	
	At 4 months	At 12 months	At 4 months	At 12 months	At 4 months	At 12 months	At 4 months	At 12 months
F_2 generation from Flemish-Polish cross	1.15½	4.1	2.9	4.12	2.4	4.3	2.2	4.14

been divided into two roughly equal groups, one containing the lighter and the other the heavier animals. For each group the average weights at 4 and 12 months respectively are shown in Table C. The result shews that ♂♂ which are lighter at maturity are also markedly lighter at 4 months, but that all the ♀♀, heavier as well as lighter, are indistinguishable in size at the earlier age. This result is strengthened by the fact that it is equally true for the Flemish-mixed and the Flemish-Polish animals. For the same class of material the bucks which are destined to become heavier start to outstrip the lighter ones at an earlier age than is the case for the does.

Inbreeding. In so far as our limited experience goes close inbreeding appears to lead to diminution in weight¹, and perhaps also to some delay in the age of maturity. Decrease in weight is most marked in the case

¹ This is contrary to the opinion of Huth ('87) who inbred rabbits for seven generations to test this point. Though the average weight of his animals at the end of his experiment was as heavy as at the beginning he does not state the numbers upon which these averages were based. Nor does he give the weights of the parents in any case. His figures do not preclude the possibility that he selected the heaviest parents in each generation to breed from, so that any effect due to inbreeding might have been masked by unconscious selection of genetic factors for increased size. That close inbreeding may be accompanied by an increase in size, provided that the largest and most vigorous animals are selected for breeding from, is brought out by the recently published experiments of Miss H. D. King ('18) with rats.

of the Flemish as is shewn graphically in Fig. 12. No doubt our original material was heterogeneous with regard to the genetic factors that

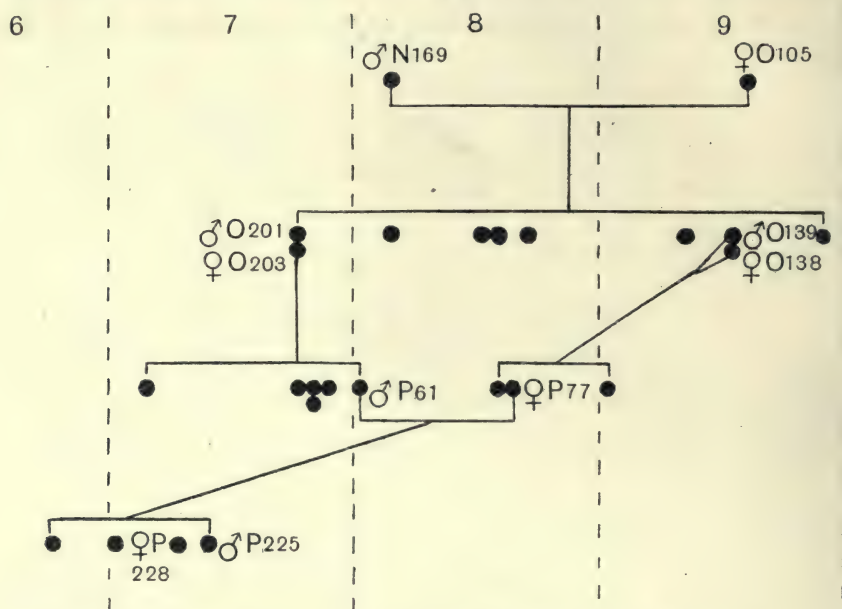


Fig. 12. Graphic representation of weight distribution in Flemish animals.
(Cf. Table I, p. 21.)

govern size, but even so the rapid diminution can hardly be accounted for by mere sifting out of genetic factors. Our experience with the Polish, though even more limited, agrees with that of the Flemish. Experiments are now in progress to ascertain the effects of continued inbreeding on weight both in these two breeds and in an extracted strain of small size from the Flemish-mixed cross (cf. Table IV, p. 24). It is hoped also in this way to obtain homogeneous material which, by a system of reciprocal crosses, can be used to elucidate the mode of transmission of size.

The experiments of which an account is given above form part of a series of investigations on heredity in rabbits for which the means have been provided out of the Fund controlled by the Development Commission.

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TABLE I.

Throughout the Tables the figure before the decimal point denotes pounds and that after the decimal point denotes ounces—e.g. 8.2 represents 8 lbs. 2 oz.

Flemish.

	Weight	Weight at 4 months		Weight	Weight at 4 months	
♂ N169	8.3	—	♀ O 105	9.10	—	} ex O 105 × N 169
♂ O 134	8.10	5.5	♀ O 136	9.15	5.7	
♂ O 135	8.12	5.5	♀ O 138	9.9 ⁽¹⁾	6.3	
♂ O 137	8.3	5.8	♀ O 203	7.13	5.5	
♂ O 139	9.9	5.11	—	—	—	
♂ O 201	7.13	4.10	—	—	—	
♂ O 202	9.6	4.15	—	—	—	
♂ O 204	8.9	5.4	—	—	—	} ex O 203 × O 201
♂ P 61	7.3	—	♀ P 56	7.15	—	
♂ P 62	7.0	—	♀ P 57	7.3	—	
			♀ P 58	7.13	—	
			♀ P 59	7.14	—	
♂ P 76	8.1	—	♀ P 77	8.11	—	} ex O 138 × O 139
♂ P 78	7.11	—	—	—	—	
♂ P 225	6.10 ⁽²⁾	—	♀ P 226	6.13 ⁽¹⁾	—	} ex P 77 × P 61
			♀ P 227	7.1 ⁽²⁾	—	
			♀ P 228	7.5 ⁽²⁾	—	

(1) Weight at 13 months.

(2) Weight at 14 months.

TABLE II.

F₁ generation from Flemish Cross.

	Weight	Maturity age in months	Weight at 4 months		Weight	Maturity age in months	Weight at 4 months	
♂ N 227	6·8	11	4·5	♀ N 230	8·3	11	4·14	} ex N 17 ⁽⁵⁾ × N 169
♂ N 228	7·4	12	4·8	♀ O 37	9·4	11	4·0	
♂ N 229	7·0	12	4·4	♀ O 39	8·6	9	4·6	
♂ N 231	6·7	12	4·0	♀ O 40	9·7	11	4·0	
♂ N 232	6·6	12	4·3					
♂ N 233	7·8	11	4·0					
♂ O 34	7·6	9	4·4					
♂ O 35	7·7	11	3·10					
♂ O 36	7·9	11	4·5					} ex N 19 ⁽⁶⁾ × N 169
♂ O 38	8·3	—	4·3					
♂ O 41	7·9	—	4·2					
♂ N 235	8·3	11	4·13	♀ O 223	7·14 ⁽⁴⁾	—	5·4	
♂ N 237	7·6	12	4·10					
♂ N 238	7·10	11	4·6					
♂ O 222	7·10 ⁽³⁾	—	4·8					
♂ O 224	6·6	9	4·10					

(3) Weight at 21 months. Weight at 9 months was=7·3.

(4) Weight at 21 months. Weight at 6 months=6·14.

(5) Weight=6·2.

(6) Weight=6·6.

TABLE III.

F₂ generation from Flemish cross.

	Weight	Maturity age in months	Weight at 4 months		Weight	Maturity age in months	Weight at 4 months	
♂ O 195	7.13	10	4.6	♀ P 75	6.11	9		
♂ O 197	6.3	10	3.13	♀ O 196	6.15	10	4.6	ex O 39 - O 41
♂ O 198	7.11	10	4.1	♀ O 200	8.6	10	3.13	
♂ O 199	7.9 ⁽⁷⁾	10	4.0					
♂ O 107	5.7	13	2.12	♀ O 106	7.6	13	3.7	ex N 230 - N 233
♂ O 109	6.2	8	3.13	♀ O 108	8.2	13	4.3	
♂ O 111	6.4	8	3.13	♀ O 110	8.3	13	3.14	
♂ O 112	5.12	13	3.7	♀ O 113	9.0	13	4.2	
♂ O 191	6.9	10	3.4	♀ O 114	8.2	10	4.1	
♂ O 192	6.0	12	3.3	♀ O 187	6.6	12	3.14	
♂ O 193	7.15	13	4.1	♀ O 189	8.2	13	4.3	
♂ P 51	6.12	10	4.6	♀ O 190	8.13	10	4.4	
				♀ P 52	7.14	12	3.4	
				♀ P 53	7.6	10	3.6	
				♀ P 54	7.6	10	3.3	
				♀ P 55	7.5	12	3.2	
♂ P 97	7.6	10	4.15	♀ P 99	5.10	12	3.7	ex N 230 - O 41
♂ P 98	6.10	12	4.3	♀ P 130	5.6	12	3.6	
♂ P 100	6.3	12	3.14	♀ P 131	7.9	12	3.11	
♂ P 127	6.4	10	4.3	♀ P 132	6.8	10	3.10	
♂ P 128	6.4	10	4.0	♀ P 134	5.7	10	3.8	
♂ P 129	6.2	10	3.15					
♂ P 133	6.8	12	4.4					
♂ P 204	5.15	9	—					
♂ P 214	6.9	11	—					
♂ P 82	6.14	12	3.14	♀ P 81	6.6	12	3.8	ex O 223 - O 222
♂ P 103	5.10	10	3.14	♀ P 83	6.5	12	3.13	
♂ P 104	6.7	10	4.1	♀ P 85	6.5	12	3.6	
♂ P 105	6.14	10	4.8	♀ P 106	6.8	11	4.0	
♂ P 107	5.15	12	4.0	♀ P 108	7.12	11	4.7	
♂ P 160	6.14	9	5.10	♀ P 159	6.12	10	5.1	
				♀ P 198	6.9	12	3.14	
				♀ P 199	5.3	9	3.6	
				♀ P 200	6.9 ⁽⁷⁾	14	4.1	
				♀ P 201	6.12	14	4.5	
				♀ P 202	6.3	10	3.12	
				♀ P 203	6.4	12	4.1	

(7) Weight at 10½ months: cf. brother, O 198, whose weight did not change between 10½—12 months.

(8) Weight at 14 months.

TABLE IV.

F₃ generation from Flemish cross.

	Weight	Maturity age in months	Weight at 4 months		Weight	Maturity age in months	Weight at 4 months	
♂ P 90	5.9	12	—	♀ P 88	5.9	12	—	} ex O 187 × O 192
♂ P 94	6.1	11	—	♀ P 89	5.13	12	—	
♂ P 121	6.0	11	3.13	♀ P 91	5.9	11	—	
♂ P 122	6.4	11	3.7	♀ P 92	6.13	12	—	
♂ P 123	5.5	11	—	♀ P 93	5.14	12	—	
♂ P 124	5.15	11	—	♀ P 120	6.15	12	3.4	
♂ P 125	4.14	11	—	♀ P 189	4.15	11	4.4	
♂ P 126	4.13	12	—	♀ P 190	6.7	9	4.4	
♂ P 192	5.3	9	3.15	♀ P 191	6.2	9	4.6	
♂ P 193	5.3	10	3.11					
♂ P 194	4.12	9	3.7					
♂ P 195	5.3	9	4.3					
♂ P 196	4.12	11	3.6					
♂ P 197	4.13	10	3.13					

F₄ generation from Flemish cross.

	Weight	Weight at 4 months		Weight	Weight at 4 months	
♂ P 233	5.2	3.0	♀ P 234	5.6	3.1	} ex P 189 × P 196

F₅ generation from Flemish cross.

	Weight	Weight at 4 months		Weight	Weight at 4 months	
♂ Q 79	—	2.8	♀ Q 78	—	2.14	} ex P 234 × P 233
♂ Q 80	—	2.14	♀ Q 81	—	2.7	

TABLE V.

Flemish-Polish cross.

	Weight	Weight at 4 months		Weight	Weight at 4 months	
♂ P 116	4.13	4.2	♀ P 115	5.9	3.15	ex O 203 × ♂ P
♂ P 117	5.2	4.1	♀ P 118	5.8	3.12	
♂ G 215	6.7 ⁽⁹⁾	—	♀ G 190	6.14 ⁽¹⁰⁾	—	½ ex O 138 × ♂ P

F₂ generation.

	Weight	Maturity age in months	Weight at 4 months		Weight	Maturity age in months	Weight at 4 months	
♂ P 223	5.4	13	3.6	♀ Q 10	4.10	11	1.6	ex P 115 × P 116
♂ P 224	4.12	13	2.12	♀ Q 11	4.13	11	1.11	
♂ Q 9	3.11	9	1.3	♀ Q 12	4.13	11	1.11	
♂ Q 14	4.2	10	1.7	♀ Q 13	4.0	10	1.10	
♂ Q 25	4.2	11	—	♀ Q 26	4.3	12	—	
♂ Q 27	4.5	10	—	♀ Q 37	3.12	10	1.14	
♂ Q 39	4.8	9	1.14	♀ Q 38	4.8	10	2.3	
♂ Q 76	3.15	8	2.3	♀ Q 75	4.8	8	2.5	
				♀ Q 77	4.5	10	1.12	
♂ P 217	4.2	12	2.8	♀ Q 3	4.10	10	2.1	ex P 118 × P 117
♂ P 218	4.12	13	2.9	♀ Q 4	4.10	10	2.0	
♂ Q 1	4.5	9	2.1	♀ Q 5	5.5	12	2.7	
♂ Q 2	4.8	9	1.11	♀ Q 6	4.1	8	2.10	
♂ Q 15	3.12	12	2.6	♀ Q 7	4.2	10	2.1	
♂ Q 18	4.8	12	2.5	♀ Q 8	4.5	12	2.3	
♂ Q 19	4.7	10	2.1	♀ Q 16	4.5	10	2.14	
♂ Q 20	4.15	11	2.12	♀ Q 17	3.15	10	2.10	
				♀ Q 21 ⁽¹⁰⁾	5.10	13	2.13	
				♀ Q 22	4.15	11	2.10	
				♀ Q 55	4.11	11	2.4	ex G 190 × G 215
				♀ Q 57	4.5	9	2.13	
♂ H 36	5.1	—	—	♀ H 39	6.10	—	—	
♂ H 37	6.7	—	—	♀ H 40	6.2	—	—	
♂ H 38	4.14	—	—	♀ H 42	6.4	—	—	
♂ H 41	4.15	—	—	♀ H 44	6.0	—	—	
♂ H 43	5.1	—	—	♀ H 45	5.9	—	—	
				♀ H 46	4.12	—	—	

(9) Weight at 10½ months.

(10) Weight at 13 months = 5.14.

,, 14 ,, = 5.11.

,, 15 ,, = 5.10.

NOTE ON THE ORIGIN OF A MUTATION IN THE SWEET PEA

By R. C. PUNNETT, F.R.S.

(With One Text-figure.)

MANY instances of the sudden appearance of new forms in plants and animals have been recorded in recent years, and speculation has been rife as to the moment at which they may be regarded as having originated. Perhaps the view most favoured is that the new form takes its origin from some abnormal division during the formation of the gametes. Nevertheless there are biologists who have placed on record their opinion that it may occur at some other stage in the life-history of the form that exhibits the new character¹. The principal difficulty in coming to any decision on this point is that in almost all cases on record the new character has not been first observed in accurately pedigreed stock. After observation it has frequently been made the subject of careful experiments in order to test its genetic nature, but this of course does not help us with the problem of its origin. Even in *Drosophila*, with its century of mutants, there does not appear to be a case where the new form can be traced backwards through definite individuals for several generations. For this reason I have thought it worth placing on record the following facts in connection with the appearance of a new form of sweet pea in pedigree cultures. The form in question is the so-called "cretin," already described by Mr Bateson and myself in an earlier number of this Journal². It is a monstrous form of which the chief characteristic is the straight stigma protruding through the

¹ See more especially Johannsen, *IV^e Conférence Internationale de Génétique*, Paris, 1911, and Emerson, *The American Naturalist*, June 1913. A good general discussion on the subject is to be found in Baur's *Einführung in die experimentelle Vererbungslehre*, 2. Aufl. 1914, pp. 288 seq.

² *Journal of Genetics*, Vol. 1. 1911.

cleft keel (cf. Fig. 1). The standard and wings are generally smaller than in the normal flower and fail to expand fully, but in these respects a good deal of variation is to be found. The cretin is however always characterised by one other feature; it is invariably sterile on the female side. The fact that this peculiar form appeared as a single individual in a pedigree culture has already been recorded. Data have also been given to shew that it behaves as a simple recessive to the normal form¹. There arises the question whether the evidence is consonant with the view that the original mutation occurred in the maturation divisions of the germ cells, or at some other stage. To attempt to answer it involves a consideration of all the details connected with the coming of the



Fig. 1. Two flowers of the original cretin plant, No. 14618/1907.
Flowers of other cretins are figured on Pl. XL, *Journ. Gen.*
Vol. I. 1911.

cretin, in so far as they are known, together with those of its subsequent behaviour.

In 1903 a cross was made between two white sweet peas, *Blanche Burpee* (long pollen) and *Emily Henderson* (round pollen)². From 3 purple F_1 plants three large F_2 families were raised in 1905. From one of these F_2 families, No. 309 containing 187 plants, the seed of 29 individuals was saved to give an F_3 generation. These 29 families were raised in 1906 and resulted in 2083 individuals all of which were normal

¹ *Journal of Genetics*, Vol. I. 1911; *ibid.* vol. III. 1913.

² Cf. *Rep. Evol. Comm. Roy. Soc.* IV. 1908, pp. 9 seq. where details will be found of the various characters entering into the cross.

(cf. Table I). From one of the F_2 families, No. 304 containing 181 plants, seed was saved from 14 individuals to give an F_4 generation. From these 14 plants there resulted an F_4 generation of 1118 individuals of which all were normal save one (cf. Table I). The exception was the original cretin which appeared in a family of 52 plants raised from the

TABLE I.

Record Nos. of F_2 plants 1906	Record Nos. of derived F_2 families 1906	No. of plants in family	Record Nos. of F_3 plants 1906	Record Nos. of derived F_4 families 1907	No. of plants in family	Record Nos. of F_4 plants 1907	Record Nos. of derived F_4 families	No. of plants in family
309 ¹	301	159	—	—	—	—	—	—
— ²	302	121	—	—	—	—	—	—
— ⁴	303	60	—	—	—	—	—	—
— ⁶	362	64	—	—	—	—	—	—
— ⁷	304	181	304⁶	146	52*	146 ⁴	144	1
— ⁸	363	35	— ⁷	147	46	— ⁸	145	6
— ⁹	305	126	— ⁸	148	87	— ⁹	146	26
— ¹⁰	306	61	— ¹⁰	149	83	— ¹¹	147	61
— ¹¹	307	67	— ¹¹	150	—	— ¹²	148	69
— ¹³	365	68	— ¹²	151	128	— ¹³	149	2
— ¹⁵	308	59	— ¹⁴	152	169	— ¹⁴	150	2
— ¹⁶	368	26	— ¹⁷	153	242	— ¹⁵	151	1
— ¹⁷	369	82	— ¹⁸	154	91	— ¹⁷	152	27
— ¹⁸	309	25	— ¹⁹	155	30			
— ¹⁹	370	21	— ²⁰	156	23		Total	195
— ²⁰	380	24	— ²²	157	90			
— ²¹	310	54	— ²³	158	29			
— ²²	381	20	— ²⁵	159	48			
— ²³	371	62		Total	1118			
— ²⁴	311	20						
— ²⁵	372	196						
— ²⁶	373	25						
— ²⁸	374	193						
— ²⁹	375	57						
— ³⁰	313	75						
— ³¹	382	39						
— ³²	376	49						
— ³³	377	57						
— ³⁹	386	57						
Total		2083						

* Family in which the cretin appeared. The record number of the cretin was 146⁵. Further details as to the nature of the F_2 , F_3 , and F_4 families will be found in *Report IV to the Evolution Committee of the Royal Society*, pp. 14, 15.

F_3 plant 304⁶/1906. This family of 52 was numbered 146 in 1907. The appearance of the cretin led to the saving of seed from normal individuals of this family, but since many of them had been pulled up before

the cretin was discovered the numbers saved were fewer than could have been wished. For various reasons the sowing of these seeds was postponed until 1912. They germinated poorly and the F_5 families from 9 plants consisted of but 195 individuals (cf. Table I). No cretin however was found among them. Seed was collected from the four plants Nos. 145-148 and sown in 1913. The 135 plants which resulted were all normal. The series of experiments was not continued beyond the F_6 generation.

The cretin then had its origin in a single seed of the F_3 plant No. 304⁶/1906 and was the only case of its kind in a family of 52 plants. None of the 13 sister plants of 304⁶ produced a cretin among a progeny of over 1000, nor did such a plant appear in the large F_3 generation of 2083 individuals of which 304⁶ was a member. Though the F_5 generation raised from the sister plants of the cretin was not large, yet four of the families were certainly of sufficient size to have produced cretins had they been heterozygous for this simple recessive character. The evidence taken together renders it unlikely that the origin of the cretin was due to the meeting of two germ cells which had each lost the normal factor. Were the mutation of germinal origin we should be inclined to place its occurrence in the parent plant of 304⁶, viz. in the F_2 plant 309⁷, and we should have expected cretins to form about 25 % of the family in which they first appeared. Again we should have looked for their further appearance in some of the F_5 families grown from sister plants of the cretin itself. We are led therefore to suppose that the appearance of this peculiar form is due to a change in the individual at some stage *after* fertilisation whereby the factor for the normal flower was either dropped out or altered during the somatic divisions.

It has been assumed that the cretin always behaves as a simple recessive, and some evidence has already been published in support of this assumption. More extended experience during the past few years has served to confirm this view. Crosses between cretins and normals of various families have been carried to the F_2 and F_3 generations and in no case is there any reason for supposing that the cretin behaves otherwise than as a simple recessive. A brief summary of the results is given in Table II. Over a period of seven years 80 families have been bred in which cretins occurred. Out of 5520 plants recorded in these families 4198 were normal and 1322 were cretins—a proportion not far removed from the expected ratio 3:1. There is therefore no reason for supposing that the difference between the cretin and the normal is other

than that of a single factor, and this, taken in conjunction with the circumstances under which it made its appearance, supports the view that the original plant arose, not through the union of two germ cells which

TABLE II.

Year when grown	No. of plants	No. of normals	No. of cretins	No. of families
1910 ¹	640	486	154	9
1912 ²	815	590	225	13
1913 ²	1026	756	270	20
1914	711	562	149	2
1915	1455	1124	331	25
1916	873	680	193	11
Totals	5520	4198	1322	80
Expectation	4140	1380	

had lost the normal factor, but through some radical alteration in the zygote after union between two normal gametes had already taken place.

¹ Recorded in *Journal of Genetics*, Vol. I. 1911, p. 295.

² Recorded in *Journal of Genetics*, Vol. III. 1913, pp. 102, 103.



ON HYBRIDISATION OF SOME SPECIES OF *SALIX*.

BY S. IKENO, F.M.L.S.

(With Plate I and One Text-figure.)

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INTRODUCTION.

THE well-known work of Max Wichura on the hybridisation of *Salix*² will ever remain the master-piece of investigations of such kind, but as its publication dates back to more than fifty years ago, and as since then no extensive researches on the hybrids of this genus have ever been undertaken by any one, except some experiments made by Kerner von Marilaun for comparing the date of first flowering of parents

¹ Some of the results contained in this paper were published in *Bot. Mag. Tōkyō*, Vol. xxx. 1916, pp. 316—320.

² *Die Bastardbefruchtung im Pflanzenreich erläutert an den Bastarden der Weiden*. Breslau, 1865.

and hybrids¹, it would not be without interest to study them from the standpoint of modern genetical science. Thus very often the view has been expressed that hybrids between various species of this genus always breed true in later generations. The objects of my experiments, which were begun in 1910 and continued till now, were to ascertain, first whether segregation of characters takes place, and secondly whether in this process, if it occurs, the Mendelian ratio can be detected. As stated below, since willows come to flowering only in their third year or even still later, the completion of the work will naturally require a very large number of years, and if I continue it, I must necessarily leave a very large space of my experiment-garden occupied by hundreds of plants for a long duration of time. Thus, some inconclusive results contained in this paper could not be brought to a definite end without the cultivation of a considerably larger number of plants, perhaps ten times as many as those already raised. This is quite impossible for the present author, who has only a small piece of land at his disposal and has yet to perform there much other work. So it was decided to discontinue the experiments on *Salix*, except as regards certain points, and to publish the results so far obtained. Although, as above noticed, the breeding experiments dealt with in this paper were commenced in 1910 they contain many imperfections, and are very far from being complete, so that this paper may be perhaps regarded as a sort of preliminary communication.

I. METHODS OF INVESTIGATIONS.

Flowers of various species of *Salix* open in Tôkyô generally at the end of February and at the beginning of March, though in some, such as *S. Caprea*, *S. triandra* var. *nipponica*, they open only at the end of April.

For the experiments of hybridisation male branches were cut off and brought to a warm room, a few days before the opening of flowers, and placed with their bases in a bottle full of water. When some flowers began to open and to shed pollen I collected the latter in a Petri dish by rubbing inflorescences with a hair pencil. As several days are wanted for the opening of all flowers in one branch I repeated the process every day till all flowers opened and began to shrivel. The Petri dish with pollen was preserved in a cold, dark place. Pollen-

¹ *Planzenleben*, Bd II. 2 Ausg. 1905, p. 510.

grains will remain effective for a very long time, as Wichura has already indicated¹.

Branches with female inflorescences were enclosed in a paper bag two or three days before their flowers began to open. When this took place the inflorescences were rubbed with a hair-pencil covered with pollen, and this process was repeated for several days till all opened flowers had been dusted with it. The paper bag was removed when the protruding stigmas of all flowers had shrivelled and there was no more danger of contamination by undesired pollen. If hybridisation succeeds the stigmas shrivel after two or three days, and the ovaries begin to swell gradually; but if not, the stigmas remain perfectly fresh for many days, and the catkins finally fall off. Fruits are generally ripe in May. Seeds were sown in a pot immediately after their collection, because, as Wichura has already shown², they very soon lose their germinating power. Seeds begin to germinate after two or three days, and seedlings grow fairly rapidly. In March of the next year they are transferred from the pot to the earth. Many of them come to flower in their third year but sometimes much later.

II. BREEDING EXPERIMENTS.

Though the hybridisations between several species of *Salix* cultivated in our Botanical Garden in Komaba near Tōkyō were performed with success³, I shall deal here chiefly with that between *Salix purpurea* var. *multinervis* (below designated simply as *S. multinervis*, Japanese name = Inukoriyanagi) and *S. gracilistyla* (Japanese name = Neko-yanagi)⁴.

These two species grow wild in the vicinity of Tōkyō, and in 1910 I found one female plant of *S. multinervis* and one male of *S. gracilistyla* cultivated in our Botanical Garden which had some time previously been transplanted from their respective natural localities. In that year I effected the hybridisation between these two plants, a process which seems to take place with great difficulty, because though I have dusted many female catkins with protruding stigmas repeatedly with an

¹ Wichura, *l. c.* p. 5.

² *L. c.* p. 6.

³ The table placed at the end of this paper will indicate all hybridisations done by me, with their respective successes and failures.

⁴ As to the scientific names of these two *Salix*-species the opinions of our systematists do not accord with each other, and I do not know whether or not the names employed in this paper are really the correct ones.

abundant quantity of pollen, the large majority of ovaries did not come to maturity, and I got only a few good seeds, which have given rise to fourteen F_1 -individuals. In 1911 the same hybridisation was repeated on the same female tree used in 1910, and I got almost fifty seedlings. The result of the latter hybridisation was entirely different from that in 1910, as described later in this paper (p. 51 ff.). The reciprocal hybridisation was not done in 1910, for the female plant of *S. gracilistyla* was not then available in our Botanical Garden. That year however I brought some branches of its female plant from a wild growing locality, some twenty miles away from Tôkyô; they were cultivated as cuttings, and came to flowering in 1912. One of these female individuals was hybridised in 1912 with the male plant of *S. multinervis*. This hybridisation failed, and no ripe seeds were obtained. In 1918 the two reciprocal hybridisations were repeated, and as I was able to obtain a certain number of seedlings from one of them, *S. multinervis* ♀ × *S. gracilistyla* ♂, my experiments will, as far as possible, be continued on these plants. The other, *S. gracilistyla* ♀ × *S. multinervis* ♂, failed again; it seems to be well-nigh impossible¹.

A. Results of the Hybridisations done in 1910.

The fourteen individuals obtained as the result of the hybridisation performed in 1910 began to flower in 1912, and were found to consist of ten females and four males which show clearly the hybrid nature in their vegetative organs as well as in their catkins.

The corresponding characters of the two parents and the F_1 plants dealt with in this paper are as follows:

S. multinervis. Stem and branches erect. Leaves glabrous on both surfaces², without stipules (cf. Text-fig. 1 B). Catkin, either male or female, sparingly hairy (Plate I, fig. 2 ♂—♀). Stigma bright scarlet-coloured.

S. gracilistyla. Stem and branches spreading. Leaves hairy on the lower surface, especially along mid- and side-veins, stipulate (cf.

¹ In both 1912 and 1918 a number of female catkins of *S. gracilistyla* were repeatedly dusted with plenty of pollen of *S. multinervis*, yet only very few ovaries in each catkin (only 1—10 out of some 400 ones in one catkin) have swollen to a certain extent, and after a few days all of these catkins have fallen off. In 1918 however only one catkin remained on the tree, and some 10 small fruits on it came to open, but were found to contain a few tiny seed-rudiments with seed-hairs normally developed.

² Except few, very young leaves yet inrolled in the bud, which are very sparingly hairy.

Text-fig. 1 A). Catkin, either male or female, densely covered with long gray hairs (Plate I, fig. 1 ♂—♀). Stigma green.

F_1 plant. Stem and branches spreading. Leaves glabrous on both surfaces, either with or without stipules. Catkin, mostly densely hairy as in the one parent (Plate I, fig. 3 ♂—♀), or sparingly so as in the other (Plate I, fig. 4 ♂—♀), according to individuals. Stigma scarlet-coloured.



Fig. 1.

Below I will compare with each other the characters above enumerated in the P -, F_1 - and F_2 -generations respectively.

(a) *Habit of Stem.*

S. multinervis has an erect stem with its branches directed upwards, as is usual in many trees, while in *S. gracilistyla* the stem ramifies near to the base into many slender branches, which are directed horizontally, and often lie upon the ground. This fact causes a great difference in the habit of the two species; the former is erect and high, while the latter is low; but as in the latter the branches spread horizontally in all directions it comes to occupy a much larger space of ground than

the former. All F_1 plants are at first exactly similar to *S. gracilistyla* in this respect, so that the spreading habit may then be considered to be dominant to the erect, but as the tree becomes older many branches which are directed upwards are produced as in *S. multinervis*, thus these older plants have become intermediate between the two parents. From these F_1 plants I obtained 442 in the F_2 generation, of which 224 are spreading and 218 erect. The fact that the erect habit is a pure recessive character against the spreading has been also proven in the following way: I fertilised an extracted erect F_2 plant by the original erect parent (= *S. multinervis*), and found the offspring, numbering 89 in all, to be *erect* without exception. The segregation of the characters under consideration in F_2 generation has thus been clearly shewn, but the ratio of the two kinds of plants is very different from what we might have expected had this segregation taken place in the usual Mendelian fashion.

(b) *Hairiness of Leaves.*

In *S. multinervis* the leaves are quite glabrous on both surfaces, whilst in *S. gracilistyla* they are at first hairy on both surfaces, and throughout their life on the lower, being more or less densely covered with long gray hairs, especially along the veins. Leaves of F_1 plants resemble the former entirely in this respect, because they are perfectly glabrous on both surfaces, so that the non-hairiness may be considered to be dominant to the hairiness¹. In the F_2 generation I got 425 plants in all, of which 351 have perfectly glabrous leaves like the one parent, and 74 leaves more or less hairy underneath like the other. It may here be remarked that among the hairy leaves the degree of hairiness is very different in different individuals, and that I was unable to find even a single plant which is so densely hairy as in the one parent, *S. gracilistyla*. This is perhaps due to the fact that we have here a large number of factors concerned in the hair-production, and it seems not unlikely that the cultivation of a much larger number of F_2 plants may give rise to a certain number of such leaves which are as hairy as in *S. gracilistyla*.

In respect to the character "hairiness of leaves" I will mention here the hybrid between *S. multinervis* and *S. viminalis*. The former has quite glabrous leaves as above stated, while the latter, which is found here as well as in Europe, has, as is well known, leaves very

¹ Some of the inner leaves in buds, and sometimes a few very small ones crowded together at the base of young branches, are more or less hairy.

densely covered underneath with long hairs of silvery lustre. The F_1 hybrids made in either of the two reciprocal ways have leaves which are always hairy underneath but much less densely so than in *S. viminalis*. In the F_2 generation from *S. multinervis* \times *S. viminalis* I was able to get only 76 plants in all. Of these 31 have leaves quite glabrous as in the one parent, whilst the remaining 45 were hairy in various degrees, and of the latter one plant was found to possess leaves which were as densely hairy as in *S. viminalis*.

The segregation of the character "hairiness" is accordingly quite evident in both cases above described, but the proportion of dominants and recessives in F_2 is very different from the usual Mendelian ratio, and reminds us of the occurrence of a complex segregation.

Here may we be allowed to make a little digression. As stated above, the leaves of F_1 hybrids between *S. multinervis* and *gracilistyla* are wholly glabrous, whilst in hybrids between the former and *S. viminalis* they are hairy underneath though less densely so than in the latter, so that these hybrids may be regarded in this respect as intermediate between the two parents. The hairy condition is apparently *recessive* in the former case, and *dominant* (or strictly speaking *intermediate*) in the latter. How such different conditions may occur in spite of the fact that we have used the same species *S. multinervis*—one and the same tree in both cases—must of course remain a matter of conjecture so long as no extensive culture of several later generations of such hybrids has been made, but the following may be perhaps one of the probable explanations based on the presence-and-absence hypothesis. Let **H** represent the factor (or the factor-complex) for the hairy condition in *S. gracilistyla* and let **I** represent the inhibitory factor contained in *S. multinervis*, then we have *S. gracilistyla* = **HHii** and *S. multinervis* = **hhII**, therefore F_1 = **HhIi**, and since the factor **I** is able to suppress wholly the hair-producing action of **H** we have in the hybrid **HhIi** leaves which are entirely glabrous. Since in the hybrids between *S. multinervis* and *viminalis* leaves are hairy in contrast to those between the former and *S. gracilistyla* we are led to think that the factor (or the factor-complex) for the hairy condition in *S. viminalis* is different from that in *S. gracilistyla*. If we represent that factor by **H'**, then we have *S. viminalis* = **H'H'ii**, *S. multinervis* = **h'hII**, and F_1 = **H'h'Ii**. The same inhibitory factor **I** which was responsible for the entire suppression of the hair-producing action of the factor **H** in *S. gracilistyla* may be regarded as being less potent against **H'** than against **H** and able to prevent the action of **H'** only partially, so that in

the hybrid H^1h^1li leaves are hairy, though less densely so than in the parent *S. viminalis*.

(c) *Stipules*.

Leaves of *S. multinervis* are exstipulate (cf. Text-fig. 1 B, p. 37), whereas those of *S. gracilistyla* are stipulate (cf. Text-fig. 1 A). The F_1 plants may be said to be a mosaic of the two parents respecting the behaviour of stipules, for each individual is always provided with both kinds of leaves, stipulate as well as exstipulate, and even in one branch those with stipules may alternate with those without them. The degree of their development is also very variable in different leaves, because they are sometimes very conspicuous (cf. Text-fig. 1 C, p. 37), sometimes very insignificant being represented by mere tiny scales; not rarely we have one unpaired stipule on one side of the leaf. In F_2 plants we see generally the same behaviour of stipules as in F_1 , for then they are provided both with stipulate and exstipulate leaves. Besides such plants we have some F_2 individuals where we could yet find no stipulate leaves, i.e. where all leaves are exstipulate exclusively¹. Thus of 232 plants examined we have 170 with both kinds of leaves and 62 with exstipulate leaves only. The latter plants are already six to seven years old, and are pretty advanced in their growth, for many of them are more than 1 metre, and some even $1\frac{1}{2}$ metre high, and are provided with a large number of branches. One might therefore be led to conclude that they really lack stipules, and are the segregates in a Mendelian sense, but I think that such a definite conclusion may yet be considered as too hasty, because I have many times experienced the fact that plants which were at first provided with exstipulate leaves only, were later found to produce some leaves which are clearly stipulate. Thus it is not unlikely that the 62 plants referred to above, in which exstipulate leaves were exclusively found so far, may in future bear some stipulate ones. And if the latter alternative really holds good, then perhaps we have here a case of the so-called "blending inheritance" or "constant intermediate inheritance" where the behaviour of stipules in F_1 , which is intermediate between that of the two parents, always repeats itself throughout later generations. But if, on the contrary, it be proved beyond all doubt that in F_2 we have a certain number of plants with exstipulate leaves exclusively, the segregation of the characters "stipulate" and "exstipulate" may be

¹ All F_2 plants were examined for stipules every year, and several times each year in different stages of the development of their branches.

considered to take place, and then we have to deal with "alternate" instead of "blending inheritance." It will be seen however from what was stated above that we are unable as yet to prove the occurrence of either the one or the other kind of inheritance. We may also here remark that so long as any one, wishing to prove the segregation of the allelomorphic characters under consideration, takes plants with exstipulate leaves for the standard, or to borrow the word of Nilsson-Ehle, for the "analyser",¹ he would never be able to arrive at a definite conclusion, owing to the possibility that plants which were at first provided with exstipulate leaves only might later develop some stipulate ones. If, on the contrary, we could find in F_2 even one plant with all its leaves provided with stipules like the one parent *S. gracilistyla* it would be possible to reach safer conclusions as to the occurrence of segregation of the allelomorphs under consideration. But not even one single such plant has been obtained till now. Probably the problem will not be definitely solved without breeding experiments conducted on a far larger scale than was possible for the present author. In short, my experiments have not been able to prove the segregation of the characters "stipulate" and "exstipulate."

(d) *Colour of Stigma.*

In *S. multinervis* the stigma is bright scarlet, while in *S. gracilistyla* it is green. In F_1 plants it is scarlet as in the former. In F_2 the segregation of the two opposite characters is quite evident. Thus we have 115 and 16 plants with scarlet and green stigmas respectively, while 7 plants have greenish-red stigmas. If we add those with scarlet and greenish-red stigmas together, we have 122 red and 16 green, i.e. almost 8 red : 1 green. The segregation of allelomorphic characters "red" and "green" is thus clear, but as in other characters hitherto enumerated the usual Mendelian ratio cannot be detected.

(e) *Character of Catkins.*

In *S. gracilistyla* the catkin, either male or female, is long and broadly cylindrical, and very densely covered with long gray hairs (Plate I, fig. 1, ♂—♀), whereas in *S. multinervis* it is much shorter and narrower, and very sparingly hairy (Plate I, fig. 2, ♂—♀). The chief difference between the catkins of these two species lies thus in the degree of hairiness: in the one they are densely hairy, while in the other they are sparingly so. The distinction between the two in this

¹ *Lunds Universitets Årsskrift*, N.F., Afd. 2, Bd VII. 1911, p. 18.

respect is sharp, and there is never found any transitional form between the two.

In F_1 I have obtained two sorts of individuals: one of them has its catkins densely covered with long gray hairs as in the one parent *S. gracilistyla* (below designated as plants of *G*-type from the word *gracilistyla*) (Plate I, fig. 3, ♂—♀), whereas the other has its catkins resembling those of *S. multinervis*, i.e. sparingly hairy (designated below as plants of *M*-type from the word "*multinervis*") (Plate I, fig. 4, ♂—♀). The distinction between the two types of catkins is generally as sharp as between those of the two original parents, though in respect to the male catkins the distinction between the two types is sometimes difficult to be made out.

Let us first describe the results obtained in the F_1 and F_2 generations. As above stated (p. 36) I had only 14 F_1 individuals, which may be classed as follows:

1. <i>G</i> -type	2. <i>M</i> -type
11	3
4 ♂ 7 ♀	0 ♂ 3 ♀

Not one single male plant of *M*-type was obtained in F_1 , but a few plants belonging to this category appeared in F_2 as stated below.

The following crosses among the F_1 plants were made in 1912 and 1913, viz.:

1. *G*-type ♀ × *G*-type ♂.
2. *M*-type ♀ × *G*-type ♂.

The fertilisation *M*-type ♀ × *M*-type ♂ was not possible then, because, as just stated, no male plant of *M*-type appeared in F_1 . In 1914, however, I got one male F_2 plant of this type resulting from the hybrid *M*-type ♀ × *G*-type ♂ above mentioned (Plate I, fig. 4 ♂), and I have done the fertilisation between this male and the female *M*-type F_1 plant used in 1912 and 1913, thus:

3. *M*-type F_1 ♀ × *M*-type F_2 ♂.

The results of these three fertilisations are as follows:

	1. <i>G</i> -type ♀ × <i>G</i> -type ♂.	
	<i>G</i> -type	<i>M</i> -type
	78 ♂ 109 ♀	2 ♂ 30 ♀
Totals	187	32
	(=85.4%)	(=14.6%)
		Total
		219

2. *M-type* ♀ × *G-type* ♂.

	<i>G-type</i>	<i>M-type</i>	<i>New type</i> ¹	Total
	33 ♂ 44 ♀	18 ♂ 49 ♀	7 ♂ 8 ♀	
Totals	77	67	15	159
	(=48.4%)	(=42.1%)	(=9.5%)	

If we add together plants of *M-type* and those of *New type*, both being very similar to each other (cf. below, p. 44) we have

	<i>G-type</i>	<i>M-type</i> + <i>New type</i>	Total
	33 ♂ 44 ♀	25 ♂ 57 ♀	
Totals	77	82	159
	(=48.4%)	(=51.6%)	

3. *M-type* F_1 ♀ × *M-type* F_2 ♂.

	<i>G-type</i>	<i>M-type</i>	<i>New type</i>	Total
	3 ♂ 0 ♀	2 ♂ 8 ♀	2 ♂ 3 ♀	
Totals	3	10	5	18
	(=16.7%)	(=55.5%)	(=27.8%)	

If we add together, as before, plants of *M-type* and those of the *New type*, we have

	<i>G-type</i>	<i>M-type</i> + <i>New type</i>	Total
	3 ♂ 0 ♀	4 ♂ 11 ♀	
Totals	3	15	18
	(=16.7%)	(=83.3%)	

If we add together all offspring derived from the three fertilisations, we have

	<i>G-type</i>	<i>M-type</i>	<i>New type</i>	Totals
1. <i>G-type</i> × <i>G-type</i> ...	187	32	0	219
2. <i>M-type</i> × <i>G-type</i> ...	77	67	15	159
3. <i>M-type</i> × <i>M-type</i> ...	3	10	5	18
Totals ...	267	109	20	396
	(=67.4%)	(=27.5%)	(=5.1%)	

¹ For description, see below, p. 44.

² This year (1918) almost all the offspring derived from this fertilisation were found to possess some buds containing young catkins, but many of the latter ceased to grow in their very young stages, having been killed by the severe frosts of last winter, so that I was able to ascertain in 18 trees only the type of catkins borne by each of them.

or, if we add plants of *M*-type and those of New type together, we have

		<i>G</i> -type	<i>M</i> -type+New type	Totals
1.	<i>G</i> -type \times <i>G</i> -type	187	32	219
2.	<i>M</i> -type \times <i>G</i> -type	77	82	159
3.	<i>M</i> -type \times <i>M</i> -type	3	15	18
	Totals	267	129	396

(=67.4%) (=32.6%)

As will be seen from the above tables the fertilisation between the *G*-type ♀ and the *G*-type ♂, which we may perhaps consider as corresponding to the self-fertilisation of a certain F_1 plant which bears hermaphrodite flowers, gives rise to many *G*-type and few *M*-type F_2 plants (cf. No. 1 in the above tables). The diametrically opposite behaviour will be seen in the fertilisation between *M*-type F_1 ♀ and *M*-type F_2 ♂ plants, for then comparatively many *M*-type and comparatively few *G*-type plants are produced (No. 3 in the above tables). In contrast to the two above cases the fertilisation between ♀ and ♂ plants belonging to the two different types gives rise to the offspring of both types in almost, though not quite, equal numbers (No. 2 in the above tables). From these experiments we see that each of the F_1 plants, whether *G*-type or *M*-type, is heterozygous, and gives rise by a fertilisation corresponding to self-fertilisation in hermaphrodites, not only to offspring of the type similar to itself, but also to a small proportion of those belonging to the other¹. The occurrence of segregation of the catkin character under consideration in F_2 is thus quite evident; it must however be remarked that the ratio of the numbers of plants of both types is then very different from the usual Mendelian one.

Before proceeding further, I must make some remarks about plants marked as "New type" in the above tables. These plants which have arisen in F_2 from either *M*-type \times *G*-type (in the ratio of almost 10%, cf. the above tables) or *M*-type \times *M*-type (in that of almost 28%), but never from *G*-type \times *G*-type, are very similar to those of *M*-type, and differ from the latter simply by the entire absence of hairs in catkins (Plate I, fig. 5, ♂—♀)². The catkins of this form appear to be much more intensely black than in plants of *G*-type or *M*-type, but this is only apparent, because though the bracts of the two latter are in

¹ With some reserve in the case of *M*-type plants, because I was not able to get male *M*-typed plants in F_1 , and have depended upon the fertilisation *M*-type $F_1 \times$ *M*-type F_2 .

² At least to the naked eye, because examined under the microscope, the bracts of catkins of this form are found to possess a few short hairs.

reality just as black as those of this new form, their colour is partly concealed by hairs covering them. In this new form there are two kinds of female plants, the one possessing red stigmas and the other green ones. In 1916, when they appeared for the first time, a few female catkins (flowers with green stigmas), which did not look quite healthy, were fertilised with pollen from the male catkins of the same type, and a few seeds were obtained, which did not germinate. This year (1918) I repeated the same fertilisation (flowers with red stigmas), and got many seeds, which were able to germinate and have given rise to a certain number of seedlings. Whether or not the latter breed true to the types of their parents is of course yet unknown; it is, nevertheless, not unlikely that this new type is a mutant—possibly a loss-mutant produced on account of the loss of the factor for the hair-formation—and arising after hybridisation. Mutation after hybridisation has, as is well known, been sometimes discovered in *Oenothera* by de Vries¹, Gates², etc., and also in *Rubus* by Lidforss³.

What I have described above about the formation of the two types of plants after hybridisation between *S. gracilistyla* and *S. multinervis* is merely the description of the results actually gained, and I must now go into their interpretation. The question is, should the appearance of both *G*-type and *M*-type offspring in F_1 be regarded as the result of segregation, or may this fact be explained in any other way? The exact answer to such a question cannot be given without further breeding experiments, and I am, for the present, only able to make certain hypotheses about it.

According to the first hypothesis one of the two original parents, either *S. gracilistyla* or *S. multinervis*, should be regarded as being heterozygous, at least in respect to the catkin character under consideration. Thus if, for instance, we denote the *G*-type character by *G* and its absence, i.e. the *M*-type one, by *g*, and if we further give to *S. gracilistyla* and *S. multinervis* the formulæ *Gg* and *gg*, respectively, we have in the fertilisation of the two *Salix*-species a back-cross $Gg \times gg = Gg + gg$, thus explaining the production of the two types of plants in F_1 . It will be of course the same, if we consider *S. multinervis* to be heterozygous and *S. gracilistyla* to be homozygous regarding this character. Is then either of the two *Salix*-species under consideration

¹ See, for instance, *Die Mutationstheorie*, Bd I, pp. 211, 212 and Bd II, pp. 425, 426; also *Gruppenweise Artbildung*, p. 302 ff.

² *The Mutation Factor in Evolution*, p. 286 ff.

³ *Zeits. f. ind. Abstammungs- und Vererbungslehre*, Bd XII, 1914, pp. 1-13.

really heterozygous in this respect? For the elucidation of this question I have made the fertilisation between male and female trees of each of the two species—the very same trees which were used in my hybridisation experiments; this fertilisation might correspond to selfing in hermaphrodites. Seeds obtained by this process were sown, and plants developed from them—70 in *S. gracilistyla* and more than 100 in *S. multinervis*—and were found to be exactly similar to their respective parents in all respects. There is therefore no reason for considering either of the two parents to be heterozygous regarding the catkin character under consideration, and the first hypothesis should be discarded.

According to the second hypothesis one sex, either male or female, of one of the two species, is regarded as heterozygous and the other homozygous in respect to the catkin character. Thus suppose, for instance, the male plant of *S. gracilistyla* to have the formula Gg , and the female GG , then the fertilisation between them which is a back-cross gives $Gg \sigma$ and $GG \varphi$, and as G is dominant over g , *S. gracilistyla* breeds always true despite the heterozygous nature of its male plant. The hybridisation of $gg \varphi$ ($= S. multinervis$) by $Gg \sigma$ ($= S. gracilistyla$) should give in F_1 the zygotes represented by Gg and gg , but as the female G -type plant should be always homozygous ($= GG$) according to our presupposition, we should have in F_1

$$Gg \sigma + gg \sigma + gg \varphi,$$

thus not one female G -typed plant should then appear, which is contrary to the fact actually seen, because I obtained seven female G -type plants (p. 42).

What will be the case, if we suppose the presence of the inhibitory factor of hairs l ? Thus, for example, suppose the male plant of *S. multinervis* $= ll$, its female plant $= ll$, *S. gracilistyla* (both male and female) $= ll$, then the hybridisation $ll \varphi \times ll \sigma$ gives in F_1 ll and ll , and since, according to our presupposition, the male M -type plant should be always homozygous ($= ll$) we should have in F_1

$$ll \varphi + ll \varphi + ll \sigma,$$

thus not one male M -typed plant should appear, which indeed accords with the fact, for I got no such plant in F_1 (p. 42). The F_2 offspring arising from the fertilisation between male and female G -type F_1 plants (i.e. $ll \varphi \times ll \sigma = ll \varphi + ll \sigma$) should however undergo in F_2 no segregation, and give G -type plants exclusively, which is contrary to

fact, because I got through this fertilisation *G*-type as well as *M*-type plants in this generation (p. 42).

Thus the hypothesis which regards one of the two sexes as being heterozygous does not accord with the facts actually observed and is untenable.

The third hypothesis is founded on what is variously called "imperfection of dominance," "reversal of dominance," or "fluctuation in dominance," because our case seems, at least, apparently very much related to that phenomenon. We have many examples of the latter in poultry according to Bateson and Punnett¹, as well as Davenport². To cite only one example from the latter author, extra-toed individuals of poultry mated with normal give extra toe only in 73% of the offspring, the remaining 27% having the normal number of toes³, yet that both kinds of the offspring are heterozygotes was proven by the fact that each of them mated *inter se* has exhibited segregation in F_2 ⁴. According to the author just named extra toe is dominant to normal, but in 27% of the offspring this dominant character was not powerful enough to exhibit itself; we have here to deal with the phenomenon which is called "imperfection of dominance," etc.

The appearance of 11 *G*-type and 3 *M*-type plants in F_1 of our *Salix*-cross would, according to this hypothesis, be due to the latter phenomenon, and the *G*-type character which is generally dominant to the other should be regarded as having failed, in the present case, to be so in $\frac{3 \times 100}{11 + 3} = 21\frac{2}{3}\%$ of the offspring. The fact that the F_1 plants, whether *G*-type or *M*-type, are heterozygotes, and undergo segregation in F_2 , has also been proven. Thus, according to the present hypothesis, the production of the two types of plants in F_1 is not to be regarded as a process of segregation.

Let us now examine whether this hypothesis explains the facts actually observed. First of all, it must be marked that what some authors regarded as the "reversal of dominance" or a phenomenon similar to it was found sometimes on further inquiry to be explained in quite another way. Thus, for instance, Coutagne⁵ and Kellogg⁶

¹ *Reports to the Evolution Committee of the Royal Society*, Report II, 1905, pp. 114—116.

² *Carnegie Inst. Washington Publ. No. 121*, 1900; *Amer. Nat.* Vol. XLIV, 1910, pp. 129—135; *Amer. Breeders' Assoc.* Vol. VI, 1911, pp. 29—32, etc.

³ *Carnegie Inst. Washington Publ. No. 121*, p. 19, Table 10.

⁴ *L.c.* pp. 20, 21, Tables 11 and 12.

⁵ *Recherches expérimentales sur l'Hérédité chez les Vers à Soie*. Thèse, Faculté d. Sciences, Lille, 1902. ⁶ *Leland Stanford Junior Univ. Publ., University Series 1*, 1908.

discovered that in crosses of some silk-worms which spin yellow and white cocoons, respectively, the dominance is variable, because in some yellow is dominant to white, while in others the reverse takes place; this is due, as the latter author thinks, to strain or individual idiosyncrasies, but Toyama¹ has proved experimentally that this phenomenon may be better explained as the effect of a mixed breed, containing recessive as well as dominant whites, than as that of individual idiosyncrasies. Almost a similar explanation is applicable to what Correns and Lock have observed in hybrids of Maize. In Maize, *alba* × *cyanea*, where blue is dominant to white Correns found in F_1 94% blue individuals and 6% white ones²; also in Maize, Moore's Concord (white) × Black Mexican (black) Lock found that black was dominant to white, but that sometimes the reverse takes place³. As first pointed out by East⁴ and afterwards by Lock himself⁵ this was due to the fact that "a supposed pure white strain" used in the hybridisation was composed in reality of a number of genotypically different individuals which, though pure for white when selfed, differ among themselves in carrying some invisible factors which react differently in the production of colour⁶. Thus we have here to deal, not with the "reversal of dominance," but with a "mixed breed," almost in the same way as in the case of the silk-worms above enunciated. It may perhaps be reasonably doubted, whether also in the so-called "reversal of dominance" in poultry we have not to deal with similar circumstances as in Maize just mentioned.

Our case in *Salix* is however somewhat different from that of Silk-worms or Maize, inasmuch as the fourteen F_1 hybrids are derived from one and the same female plant fertilised by pollen taken also from one and the same male plant, so that if our case were really explicable on the basis of the hypothesis founded on the reversal of dominance it must necessarily follow that the *G*-typed catkin is sometimes dominant, sometimes recessive to the *M*-typed one in the same individuals, which does not seem very probable. It appears to me much more reasonable to consider that though either one of the two types of catkins, for instance the *G*-typed one, is in reality always dominant to the other,

¹ *Zeits. f. ind. Abstammungs- u. Vererbungslehre*, Bd VII. 1912, pp. 252—288.

² *Bibliotheca Botanica*, Heft LIII. 1901, pp. 53—55.

³ *Ann. R. Bot. Garden Peradeniya*, Vol. III. 1906, pp. 117—129.

⁴ *The Connecticut Agric. Exp. Station*, Bull. 167, 1912, pp. 57—100.

⁵ *Ann. R. Bot. Garden Peradeniya*, Vol. V. 1912, pp. 257—264.

⁶ Lock, *l. c.* p. 257.

its *apparent recessiveness* is caused in some cases by the influence of other factors contained in them, especially some invisible factors, an explanation similar to that first proposed by East about the Maize-cross just cited. Let me then describe below my views regarding this question. Suppose that either one of the two parents under consideration, *S. multinervis*, for instance, carries some such factors in a heterozygous condition¹. In gametic formation the latter will undergo segregation, so as to give rise to gametes containing different combinations of invisible factors. In the fertilisation between male and female plants of this species gametes differing in respect to invisible factors may come to copulation, yet the offspring will always breed true to their parent type, at least all of them will agree in their catkin character, because since the factors for the latter character are in the same homozygous condition in all of them, there will be no reason why the catkin belonging to any other than the *M*-type will come to development, so that in this case the difference of invisible factors in different offspring will be perfectly indifferent towards the development of this character.

Quite different results may however be expected in the hybridisation *S. multinervis* \times *S. gracilistyla*. All F_1 hybrids will agree now in carrying the same factors concerning the catkin character in the same heterozygous condition, for instance Gg^2 , while they will differ among themselves in containing invisible factors differently combined, just as in the former case. It is then reasonable to consider that these invisible factors, owing to the difference in the mode of their combinations in the various offspring, will co-operate with Gg , so as in the one case to let G dominate over g , and in the other to induce just the contrary effect, thus producing, respectively, the *G*- or the *M*-type in different individuals. In short, whether the one or the other type of catkins will make its appearance, may be regarded as being due to the influence of invisible factors accompanying the catkin factors. Thus if our view be true, the phenomenon seen in F_1 is to be regarded as being due to the segregation of invisible factors, but not to that of the

¹ Factors may be present, which do not by themselves alone produce any visible effect, or at least can produce some effects which are so insignificant as to escape our eyes. Such factors are "invisible ones" which are able to produce visible effects only in co-operation with other ones, either visible or invisible. Further, it is here supposed that only one of the parents carries invisible factors, but it will make no difference whatever in our logic, if we consider them to be carried by both parents.

² $G = G$ -type, $g =$ absence of $G = M$ -type; the factorial composition may really be much more complex, but it is here so represented for the sake of simplicity.

catkin factors themselves, because the latter are retained in the same heterozygous condition in all F_1 offspring. What we have seen in F_2 (pp. 42—43) is however the segregation occurring on account of the heterozygosity of the catkin factors carried by F_1 plants.

Let us now go to F_2 . As already described (pp. 42—43) the fertilisation G -type \times G -type gives rise to many G -type and few M -type plants, and the M -type \times M -type gives rise to many M -type and few G -type ones, whilst in the M -type \times G -type plants of both types are produced in almost, though not quite, equal numbers. The explanation of this peculiar mode of F_2 -segregation will, as I think, naturally follow from our hypothesis adopted about F_1 plants. We have supposed (p. 49) that each of the F_1 plants, whether G -type or M -type, possesses a similar factorial constitution in respect to the catkin character, which we have represented by Gg ; in F_2 we should have then on account of the segregation

$$GG + nGg + gg$$

in all cases, n being any positive integer equal to or greater than 2.

As already noticed (p. 49) each of the G -type F_1 plants carries besides the factors Gg a certain combination of invisible factors which we may for instance call X , and which acts together with the latter, so as to give rise to G -type catkins exclusively; accordingly all the F_2 offspring derived from the fertilisation G -type \times G -type will contain X ; and so of the F_2 plants $GG + nGg + gg$, GG and nGg (the latter under the influence of X) should be G -typed, whilst only gg should be M -typed, thus explaining the fact that the F_2 offspring consist largely of G -type plants.

On the contrary, as each of the M -type F_1 plants carries besides Gg a combination of invisible factors which we may call Y , and which acts together with the latter, so as to give rise to M -type plants exclusively, we may, by similar reasoning as above, come to the conclusion that of the offspring $GG + nGg + gg$ derived from the fertilisation M -type \times M -type, nGg (under the influence of Y) and gg should be M -type, and only GG , G -type, thus explaining the fact that the F_2 offspring are then largely M -type.

In the fertilisation M -type \times G -type we have to deal with the two kinds of combinations of invisible factors, X and Y , which are provided with diametrically opposite characters. It is clear that in F_2 some offspring will receive X , whilst some others Y , and that then Gg accompanied by X will be G -types, whilst other Gg accompanied by Y , will be

M-types. In other words, of the F_2 offspring **GG** and **gg** will belong to *G*-types and *M*-types, respectively, whilst **Gg** will belong partly to the one and partly to the other. Thus the number of plants of both types should be theoretically equal to each other, which accords, as we have already seen, with the fact really observed.

This explanation of the behaviour of our cross in F_1 and F_2 is naturally mere hypothesis which needs to be subjected to experimental verification. The latter would be however extremely difficult, if not absolutely impossible, but I intend to continue my work in this direction, as far as I can.

To summarise, the formation of the two types of catkins in F_1 is not to be looked upon as the result of segregation of the catkin character; the occurrence of the latter process in F_2 has however been clearly proven, though the ratio of the two types produced in each of the three kinds of fertilisation is quite different from what we might have expected in usual Mendelian cases.

B. Results of the Hybridisations done in 1911.

As already stated (p. 36) I have repeated in 1911 the same hybridisation done in 1910. The female plant was the very same tree used in 1910; whether or not the male plant was just the same as that used in 1910 is now unknown, but it belongs, at least, to the same vegetative line (in the sense of Fruwirth) or the same clone (Webber) as the latter, because in 1911 all male plants of *S. gracilistyla* in our Botanical Garden were exclusively derived from the cuttings of the same plant used in 1910. This hybridisation succeeded pretty well, and I got nearly fifty seedlings. They were, however, contrary to the result of the hybridisation in 1910, not hybrids at all, at least externally. They were nothing but *S. multinervis*, and when they came to flowering, all of them have proven, to my great astonishment, to be female individuals without exceptions, or in other words, the offspring were of purely *maternal* type, so-called *false* (Millardet) or *unilateral hybrids* (de Vries).

The production of either purely paternal or maternal plants as the results of hybridisation—what Bateson calls “Monolepsis”¹—has been sometimes met with by various authors. Thus Gärtner² obtained from the hybridisation *Melandrium rubrum* ♀ × *Silene noctiflora* ♂ only

¹ Report to the Evolution Committee of the Royal Society, Report I, 1902, p. 155.

² Versuche und Beobachtungen über die Bastarderzeugung im Pflanzenreich, Stuttgart, 1849, p. 37.

two hybrids and many *M. rubrum*, i.e. plants of purely maternal type. In some species of *Fragaria* Millardet¹ got plants of both paternal and maternal types by hybridisation—the well-known “hybridation sans croisement” or “fausse hybridation².” This was repeated on *Fragaria virginiana* ♀ × *F. elatior* ♂ by Solms-Laubach, who got hybrids of purely paternal type, thus confirming the results of the French author³. The same kind of hybrids as the latter were found recently by Collins and Kempton in the Gramineae *Tripsacum dactyloides* ♀ × *Euchlaena mexicana* ♂, which breeds true in later generations—what these authors call “Patrogenesis⁴.”

Hybrids of purely maternal type were obtained by Hurst from the Orchid *Zygopetalum Mackayi* fertilised by some species of *Odontoglossum*, *Oncidium* and *Lycasta*⁵.

A very detailed study was made by Lidforss on a great number of species of *Rubus*⁶. According to him the hybridisations, *R. acuminatus*, *R. divergens*, *R. dissimulans*, *R. plicatus* as females by *R. caesius* as male, and *R. polyanthemus* as female by *R. Bellardi* or *radula* as males, for instance, have given genuine and false hybrids in nearly equal numbers, whilst the hybridisations, *R. polyanthemus*, *R. insularis*, *R. Lindenbergii* as females by *R. caesius* as male have given rise exclusively to false hybrids. The false hybrids of Lidforss were always of purely maternal type, and were found to breed true in later generations.

To cite another example, de Vries obtained by the hybridisation *Oenothera Lamarckiana* ♀ × *O. biennis* ♂ false hybrids of paternal type, which were found to breed true in later generations⁷. The same hybridisation carried on in the New York Botanical Garden has given quite different results, because it has given rise to four distinct types of hybrids (MacDougal, Vail, Shull and Small)⁸, and the latter four authors came to the conclusion that the difference of their results from those of de Vries might be due to the influence of some factors, such as individual qualities as well as external conditions. It might however

¹ *Mémoire de la Soc. des Sciences physiques et naturelles de Bordeaux*, 4 Série, tom. iv. 1894, pp. 347—372.

² Besides *Fragaria* false hybrids were observed by Millardet in *Rubus* (l.c.) and *Vitis* (*Revue de Viticulture*, tom. xvi. 1901, the original paper not seen, reviewed in Winkler, *Prog. Rei Bot.* Bd ii. 1908, p. 344.

³ *Bot. Zeit.* i. Abt. Jahrg. 65, 1907, p. 53.

⁴ *Journ. of Heredity*, Vol. vii. 1916, pp. 106—118.

⁵ *Journ. R. Hort. Soc.* Vol. xxix. 1900, pp. 104—106.

⁶ *Zeits. f. ind. Abstammungs- und Vererbungslehre*, Bd xii. 1914, pp. 1—13.

⁷ *Die Mutationstheorie*, Bd ii. p. 31, and *Gruppenweise Artbildung*, pp. 156—159.

⁸ *Carnegie Inst. Washington Publ.* No. 24, 1905, p. 17 ff.

be asked, whether the *Oenothera*-species used by these authors had genotypic constitutions exactly similar to those used by the Dutch botanist. If this be really true, then this hybridisation seems to have some resemblance to what I have seen in *Salix*-cross under consideration.

My results on the hybridisation *Salix multinervis* × *Salix gracilistyla* agree with those recorded by Lidforss for *Rubus*, inasmuch as I have also obtained both genuine hybrids as well as plants of purely maternal type. The difference between the two hybridisations lies only in this: while Lidforss obtained both real and false hybrids in one and the same year. I was able to obtain them in different years (1910 and 1911). That the difference of results in these two years is due to the genotypic differences of male and female plants used by me may be absolutely denied, for, as above stated, the female plant used in both cases was one and the same tree, and the male plant in 1911 was either the same with, or at least derived in a vegetative way from, that used in 1910. As I obtained different results in different years, one might be disposed to think that whether the offspring will be real hybrids or of purely maternal type is dependent on external conditions, which indeed may be true. But I think it equally likely that our plant contains two kinds of eggs, giving genuine and false hybrids, respectively, just as we have in *Thalictrum purpurascens*¹ and some species of *Hieracium*² two distinct kinds of eggs, i.e. those which can develop only after having been fertilised, and those which are able to develop parthenogenetically, though the final decision of this question will be of course impossible without performing further breeding experiments.

Now some remarks about the sex of false hybrids. *Rubus* is always hermaphrodite, so that both real and false hybrids are naturally of this nature. The sex of those of *Melandrium* obtained by Gärtner is unknown, for he has stated nothing about it³. Those of *Fragaria virginiana* ♀ × *F. elatior* ♂ were either male or female⁴. Those of *Salix* got by me, almost fifty in number, were female without a single exception, so that they may be well said to be of *maternal type in the most strict sense of the word*, for even the sex has been inherited. Furthermore, that our false hybrids will breed true in later generations like

¹ Overton, *Bot. Gaz.* Vol. xxxiii. 1902, pp. 363—374; *Ber. d. Deutsch. Bot. Ges.* Bd xxii. 1904, pp. 274—283.

² Ostenfeld, *Botanisk Tidsskrift*, Bd xxvii. 1906, pp. 225—250; *Zeits. f. ind. Abstammungs- u. Vererbungslehre*, Bd iii. 1910, pp. 241—285.

³ L.c.

⁴ Solms-Laubach, l. c. p. 53.

those of *Rubus*, is highly probable, though not yet actually proven. This problem will be one of the objects of my future study.

That in the case of *Salix* the formation of false hybrids is due neither to parthenogenetic development of the oosphere nor to the vegetative production of embryos from nucellar cells is quite evident in view of the fact that female inflorescences covered with paper bags were never able to bear even single fruits. Many authors think¹ that in the formation of false hybrids pollen has nothing to do with fertilisation, but acts merely by irritating egg-cells in some way and enables them to develop into embryos without being fertilised; such process is called *pseudogamy*, a word first proposed by Focke². Giard³ thinks that false hybrids of purely paternal type are derived from maternal cytoplasm with the male nucleus alone, the female nucleus degenerating (Merogony!), and that those of purely maternal type are derived by pseudogamy, some stimulus to development being given by the pollen-tube without entrance of the sperm-nucleus into the egg. All these are however mere hypotheses which are simply more or less probable, and which ought to be proven cytologically. The only cytological investigation on false hybrids of plants is that of Strasburger on *Fragaria*⁴, which did not confirm the hypothesis of Giard above stated. Thus in the hybrid *F. virginiana* ♀ × *F. elatior* ♂ the former author could observe no degeneration of the egg-nucleus, while, on the contrary, not only was he able to see clearly the fusion of the sperm- and the egg-nuclei, but he was able to count in the mitosis of the fusion-nucleus the diploid number of chromosomes.

False hybrids have also been observed in animals, and there is a series of papers concerning hybrids of purely maternal type, though they never reached the adult stage. In these hybridisations, or heterogeneous fertilisation, as it is often called, eggs of the Echinoids (as *Sphaerechinus*, *Strongylocentrotus*, *Echinus*, *Arbacia*, etc.) were fertilised by sperms of the Echinoids, the Crinoids (as *Antedon*), the Mollusks (as *Mytilus*), the Vermes (as *Chaetopterus*). Since in these hybridisations the systematic affinity of the two parents is always very remote from each other, only the larvae, in more or less advanced stages of their development, were obtained, and these have always proven to be

¹ For instance, Hurst, *l.c.* p. 106; Winkler, *l.c.* p. 333.

² *Die Pflanzenmischlinge*, Berlin 1881, p. 525.

³ *Comptes-rendus de la Soc. de Biologie, cinquantième de la Soc.* 1899, Vol. jubilaire, p. 665; *Comptes-rendus hebdomad. des Séances de la Soc. de Biologie de Paris*, Vol. LV. 1903, p. 779 (original not seen; cited according to Solms-Laubach, *l.c.* p. 53).

⁴ *Histol. Beiträge*, Heft VII. 1909, pp. 43—46.

of *purely maternal type* (Vernon, Herbst, Kupelwieser, Godlewski jun., Baltzer, Morgan, etc. etc.). Cytological investigations of these false hybrids were made also by many authors. The fact has been revealed that in the hybridisation of the Echinoids the spermatozoa always enter the egg-cytoplasm. The behaviour of the sperm-nucleus was however found always not to be the same. For instance, in some cases (as *Echinus* ♀ × *Mytilus* ♂, *Strongylocentrotus* ♀ × *Mytilus* ♂) the egg- and the sperm-nuclei do not come to real fusion, and the latter undergo gradual degeneration (Kupelwieser¹); in many other cases both nuclei fuse to each other, and in later stages the paternal chromatin is eliminated in some way (Baltzer², Kupelwieser³, Godlewski jun.⁴). According to Baltzer⁵, in the hybrid *Sphaerechinus* ♀ × *Strongylocentrotus* ♂, which is intermediate between the two parents, no chromatin elimination takes place, whilst in *Strongylocentrotus* ♀ × *Sphaerechinus* ♂, which is of purely maternal type, he was able to observe the elimination of paternal chromatin during the first cleavage.

On the contrary, in the hybrid *Echinus* ♀ × *Antedon* ♂, which is of purely maternal type, and where also the sperm- and egg-nuclei fuse with each other, no elimination of paternal chromatin was observed (Godlewski jun.⁶); the latter author has also observed that the chromosomes derived both from the egg- and the sperm-nuclei participate in the first mitosis of the egg (first cleavage), though he could not there distinguish paternal and maternal chromosomes from each other. Baltzer⁷, who confirms in general the statement of Godlewski jun., was able even to distinguish both kinds of chromosomes. The results of the cytological studies of Strasburger on *Fragaria* above cited are thus in exact accordance with this discovery of Godlewski jun. and Baltzer on *Echinus* × *Antedon*, because in both cases no elimination of paternal chromatin succeeding the fusion of the two nuclei does take place. What cytological phenomena will occur in the formation of false hybrids of *Salix* should be one of the objects of our future study.

¹ *Archiv f. Entwicklungsmechanik der Organismen*, Bd xxvii. 1909, pp. 434—462.

² *Archiv f. Zellforschung*, Bd v. 1910, pp. 497—621.

³ *Ibid.* Bd viii. 1912, pp. 352—395.

⁴ *Archiv f. Entwicklungsmechanik*, Bd xxxiii. 1912, pp. 196—254.

⁵ *L.c.*

⁶ *Archiv f. Entwicklungsmechanik*, Bd xx. 1906, pp. 574—643.

⁷ *L.c.*

III. CONCLUSIONS.

After the appearance of the work of Wichura the view prevailed that hybrids between various species of *Salix* breed true in later generations. My hybridisation experiments conducted on a few *Salix*-species have shown that this is not true, at least in respect to certain characters. According to these experiments the erect habit of stem is dominant to the spreading, the hairy character of leaves is dominant to the non-hairy in one case, and recessive in another, red stigma is dominant to green, and all these characters were found to exhibit segregation in F_2 generation. Hybrids between plants with stipulate and those with exstipulate leaves exhibit a mosaic character, for some leaves have stipules and others none; the occurrence of segregation of this character in F_2 is not yet proven.

In the hybridisation *S. multinervis* \times *S. gracilistyla* the so-called *G*-type and the *M*-type offspring, differing in catkin character, appear in F_1 . This phenomenon has not yet been explained beyond all doubt, and various hypotheses have been proposed for it. Of the latter the most probable is that which supposes that either one of the parents (or both) is heterozygous in some invisible factors; the offspring derived from the hybridisation under consideration will then carry them in different combinations, and this genotypic difference will influence the factors concerning the catkin character, so as to give rise in some cases to the *G*-type, and in others to *M*-type. Thus the appearance of the two types of catkins in F_1 is not due to the segregation of the catkin factors themselves, for all F_1 plants will agree in carrying the latter in the same heterozygous condition. Their real segregation was found to take place first in F_2 ; the peculiar mode of this latter process has been explained on the basis of the hypothesis adopted in the case of F_1 plants.

The segregation of many allelomorphic characters has thus been conclusively proven, but in every case the proportion of individuals bearing each antagonistic character is very different from 3:1, 15:1, 63:1, etc. etc., usually seen in Mendelian hybrids. It would not however be surprising that I was unable to demonstrate the usual Mendelian ratios in *Salix*-hybrids, because neither Lotsy¹ nor Wichler² was able to

¹ Zeits. f. ind. Abstammungs- und Vererbungslehre, Bd VIII. 1912, pp. 325—333; IV^e Conférence internationale de Génétique, 1913, pp. 416—428.

² Zeits. f. ind. Abstammungs- und Vererbungslehre, Bd x. 1913, pp. 175—232.



Fig. 1. *Gracilistyla* ♂ — ♀.



Fig. 2. *Multinervis* ♂ — ♀.

Fig. 4. *M*-type ♂ = ♀



Fig. 3. *G*-type ♂ = ♀.

Fig. 5. Mutant(?) ♂ = ♀.

discern them in species-hybrids of *Antirrhinum* and *Dianthus*, which were studied in detail from the standpoint of modern genetic science, though there were some rare exceptions in the former genus. It is very likely that in such cases a great number of factors are concerned in the development of each character, and consequently a complex segregation takes place in F_2 , though it is equally undeniable that this segregation may be subject to some law other than Mendelian, hitherto unknown to us.

The plants arising as the results of the hybridisation done in 1910 between *S. multinervis* ♀ × *S. gracilistyla* ♂ were real hybrids, but those produced from the hybridisation done in 1911 between the same male and female trees were the so-called "false hybrids" of purely maternal type because they were nothing but *S. multinervis*. They have not yet been proved to breed true, though this is probable. All false hybrids thus obtained were of one sex, namely female. Cytological investigations are necessary here.

In conclusion, I wish to thank Mr S. Nohara, Mr Y. Tanihara and Mr M. Andô, who were very helpful to me during the present investigation.

EXPLANATION OF PLATE I.

Some of the figures are photographs by Dr Kominami, to whom my thanks are due. All slightly reduced from natural size.

Fig. 1. *Salix gracilistyla*. Above ♂, below ♀.

Fig. 2. *Salix multinervis*. Above ♂, below ♀.

Fig. 3. *Multinervis* × *gracilistyla* F_1 , *G*-type. Left ♂, right ♀.

Fig. 4. *Multinervis* × *gracilistyla* F_1 , *M*-type. Left ♂, right ♀.

Fig. 5. Mutant(?) arising from the fertilisation *M*-type × *G*-type. Above ♂, below ♀.

Table showing Successes and Failures of Hybridisations of Various Species of *Salix*.

Successes	Failures
<i>Purpurea</i> ♀	<i>Purpurea</i> ♀
× <i>Purpurea multinervis</i> ♂	× <i>Purpurea sericea</i> ♂
× <i>Gracilistyla</i> ♂	× <i>Opaca</i> ♂
× <i>Viminalis</i> ♂	× <i>Triandra nipponica</i> ♂
<i>Purpurea multinervis</i> ♀	<i>Purpurea sericea</i> ♀
× <i>Purpurea sericea</i> ♂	× <i>Purpurea multinervis</i> ♂
× <i>Gracilistyla</i> ♂	× <i>Gracilistyla</i> ♂
× <i>Opaca</i> ♂	× <i>Triandra nipponica</i> ♂
× <i>Viminalis</i> ♂	× <i>Viminalis</i> ♂
<i>Gracilistyla</i> ♀	<i>Gracilistyla</i> ♀
× <i>Purpurea</i> ♂	× <i>Purpurea multinervis</i> ♂
<i>Viminalis</i> ♀	<i>Opaca</i> ♀
× <i>Purpurea</i> ♂	× <i>Purpurea multinervis</i> ♂
× <i>Purpurea multinervis</i> ♂	× <i>Gracilistyla</i> ♂
	× <i>Triandra nipponica</i> ♂
	× <i>Viminalis</i> ♂ ¹
	<i>Caprea</i> ♀
	× <i>Purpurea multinervis</i> ♂
	× <i>Opaca</i> ♂
	× <i>Triandra nipponica</i> ♂
	× <i>Viminalis</i> ♂
	<i>Triandra nipponica</i> ♀
	× <i>Caprea</i> ♂

¹ Seeds from this cross were obtained by Mr Nohara.

STUDIES OF INHERITANCE IN THE JAPANESE *CONVOLVULUS*.

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(With Plate II and One Text-figure.)

[*Note.* In the present state of the postal service it has not been possible to submit this paper to the author for revision. EDD.]

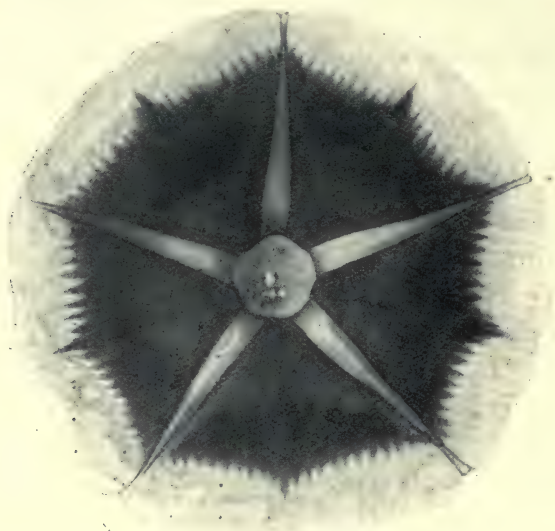
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INTRODUCTION.

THE Japanese *Convolvulus*, closely related to the Morning Glory of the Americans and known under the popular name "Asagao¹," is very extensively cultivated here since immemorial time as an ornamental plant, and contains an abundant number of races which are characterised by remarkable variation in the form and colour of leaves as well as flowers. As I have been studying the hereditary behaviour of several characters in this species for some years, and have reached definite conclusions in some respects, I am going to publish here the results of these investigations. All experiments contained in this paper were conducted in my garden in Yokohama.

The inheritance of the Japanese *Convolvulus* has already been studied by three authors, Tanaka², Toyama³ and Takezaki⁴. Of these I will speak below only about the investigations of Takezaki, some of whose



Corolla with "hukurin," seen from above.

¹ This plant has been variously called by our systematists *Ipomoea hederacea*, *Pharbitis hederacea*, *P. Nil*, etc., and I am not able to decide myself which name is really the right one.

² *Idengaku Kyôkwasyo* (A text-book of Genetics in Japanese), Tôkyô, 1915, pp. 32 ff. and 96 ff.

³ *Nippon Ikusyugakukwai Kwaihô* (Journal of the Japanese Breeders' Association), 1. 1, 1916, pp. 8, 9.

⁴ Ditto, pp. 12, 13 with many tables.

results are in agreement with mine. According to him the green colour of leaves behaves as dominant towards the yellow (*chlorina*), and in F_2 the ratio of green and yellow plants is 3:1. The genetic behaviour of flower colour is very complex, but if we classify plants simply into those with coloured and those with white flowers, white is recessive, and in F_2 the ratio of the two kinds of plants is 3:1. In some of these coloured flowers the corolla is white at its margin, so as to form a ring-shaped white patch (see the text-fig.).—what Japanese gardeners call the “hukurin”. Takezaki studied the inheritance of white-margined flowers, and found that the “hukurin” is produced by a special factor acting as a white dominant at the margin of the corolla so that the hybrid between a race with white-margined flowers and another with fully-coloured ones was found to produce the former kind of flowers in F_1 and to segregate in F_2 into the ratio 3 white-margined:1 fully-coloured. Moreover, he reported that in certain cases there is even a factor which inhibits the action of that producing the “hukurin” part.

EXPERIMENTS.

The plants originally used in my experiments are characterised as follows:

A. Leaf is yellow² (*chlorina*) (Pl. II, fig. 6), and flower white, though its throat is tinged with extremely light magenta (Pl. II, fig. 2).

B. Leaf is green (Pl. II, fig. 5), and flower dark-red³ (Pl. II, fig. 1).

These two parents were cultivated for two years before my experiments had begun, and since then this cultivation has been continued during five years. Both of them were found during cultivation to breed true entirely to their respective types.

In 1913 I performed the hybridisation between these two plants in both reciprocal ways, and in 1914 three individuals from each were grown for the purpose of further experiments.

(a) F_1 Generation.

Leaf was green: that is, green is dominant to yellow. Flower-colour was entirely different from that of either parent, and was light

¹ I shall sometimes use this word to indicate such a white patch.

² The word “yellow” is used always in this paper for brevity’s sake, but naturally it means yellowish green.

³ This colour corresponds nearly to No. 42 (Rouge) of the “Code des Couleurs” by Klincksieck and Valette, Paris, 1908.

magenta¹. The corolla is not however fully coloured, and it is white at its margin not wholly, but only near each of the five notches of its limb. Such white patch is also called "hukurin," and the words "hukurin" and "white-margined" used below refer always to flowers which are edged with white partially in such way. Both reciprocal hybrids were entirely similar to each other (Pl. II, fig. 3).

(b) F_2 Generation.

The mode of segregation of flower-colour in F_2 is rather complex. Not only are there found flowers of white, dark-red, and magenta colour exactly similar to that of the two original parents and the F_1 plant, respectively, but we have also those of scarlet colour (Pl. II, fig. 4), and in each of these colours—dark-red, magenta, and scarlet—there are three gradations of their intensity, sharply distinguishable from each other. The detailed study of the segregation of flower-colour is now under way, and will be dealt with in a future paper. For the present time, for simplicity's sake, I will call magenta and scarlet simply by the collective name *red*, and make no distinction of the intensities of colour just noticed.

The details of the segregation of leaf- and flower-colour in F_2 are shewn in Table I.

TABLE I.
 F_2 generation.

Leaf-colour	Flower-colour	White-margined or fully-coloured	$A \times B$				$B \times A$				Grand totals
			a^1	b	c	Totals	d	e	f	Totals	
green	red	white-margined	20	7	46	73	9	4	48	61	134
		fully-coloured	6	4	16	26	5	1	20	26	52
	dark-red	white-margined	9	2	29	40	5	5	22	32	72
		fully-coloured	4	0	15	19	2	5	10	17	36
	white	...	15	4	23	42	11	5	36	52	94
yellow	red	white-margined	5	5	27	37	3	8	30	41	78
		fully-coloured	1	2	14	17	2	2	14	18	35
	dark-red	white-margined	0	0	0	0	0	0	0	0	0
		fully-coloured	0	0	0	0	0	0	0	0	0
	white	...	4	2	13	19	1	1	11	13	32
Totals			64	26	183	273	38	31	191	260	533

We will consider now leaf-colour, "hukurin" and flower-colour separately.

¹ This lies between No. 566 and 571 (Violet rouge) of the "Code des Couleurs."

² The letters $a-f$ in the Tables indicate the different individuals of the 6 F_1 plants which were bred from.

1. *Leaf-colour.*

The results of my investigation are in perfect accord with those of Takezaki (p. 61), and it will be readily seen from Table II that here the segregation occurs in the simplest Mendelian fashion.

TABLE II.

F_1 plants	Results			Expected		α^1	δ^2
	Green	Yellow	Totals	Green	Yellow		
$A \times B$ ($a + b + c$)	200	73	273	204.75	68.25	± 4.75	± 7.150
$B \times A$ ($d + e + f$)	188	72	260	195.00	65.00	± 7.00	± 6.982
Totals	388	145	533	399.75	133.25	± 11.75	± 9.997

2. "*Hukurin.*"

As before stated, in spite of the fact that neither the one nor the other of the parents shows externally any sign of the "*hukurin*," this character appears in the F_1 plants, and moreover, it will be seen from Table III that in F_2 the ratio of plants with white-margined and those with fully-coloured flowers is 3:1. As of course we cannot distinguish between the white-margined and the non-white-margined condition in perfectly white flowers, plants with the latter kind of flowers are not included in this Table.

TABLE III.

F_1 plants	Results			Expected		α	δ
	White-margined	Fully-coloured	Totals	White-margined	Fully-coloured		
$A \times B$ (a)	34	11	45	33.75	11.25	± 0.75	± 9.186
" (b)	14	6	20	15.00	5.00	± 1.00	± 1.937
" (c)	102	45	147	110.25	36.75	± 8.25	± 5.250
$B \times A$ (d)	17	9	26	19.50	6.50	± 2.50	± 2.208
" (e)	17	8	25	18.75	6.25	± 1.75	± 4.165
" (f)	100	44	144	108.00	36.00	± 8.00	± 5.196
Totals	284	123	407	305.25	101.75	± 21.25	± 8.736

From the above table we see that the number of plants with white-margined flowers really obtained is always smaller than might be theoretically expected, except in $A \times B(a)$. We have however to make here the two following remarks. In the first place, the area of the "*hukurin*" part was very variable according to individuals, notwithstanding the fact that all plants were grown under exactly similar conditions. Thus not rarely the "*hukurin*" was represented by very

¹ Deviation from the theoretical number.² Standard error.

insignificant white spots in the five notches of the corolla; moreover, even in one and the same individual, which has very slightly white-margined flowers, I was able to discern the "hukurin" sometimes clearly but sometimes not at all, according to different stages of their development, so that it would not be improbable that some plants with such very slightly white-margined flowers were erroneously entered as being without them. Secondly, I have learned by experience that the mode of cultivation has great influence over the production of the "hukurin." Plants were generally grown in a field, but some of them were cultivated in pots, for example a certain number of (*c*) and (*f*) in Table III. The difference of the results due to the method of cultivation will be explained by reference to Table IV.

TABLE IV.

F ₁ plants	Cultivated in field			Cultivated in pot		
	White-margined	Fully-coloured	Totals	White-margined	Fully-coloured	Totals
<i>A</i> × <i>B</i> (<i>c</i>)	38	13	51	64	32	96
<i>B</i> × <i>A</i> (<i>f</i>)	53	19	72	48	24	72
Totals ...	91	32	123	112	56	168
Percentage	73.98	26.20		66.66	33.33	

As will be seen from the above Table, while in the field culture plants with white-margined flowers and those with fully-coloured ones are 74 and 26, respectively, i.e. are almost exactly in the ratio 3 : 1, in the pot culture there are 67 and 33, respectively, i.e. the number of plants with white-margined flowers is relatively much smaller in the latter case than in the former. Plants in (*a*), (*b*), (*d*), and (*e*) were all cultivated in the field, and we see that here the ratio of the two kinds of plants is nearly equal to 3 : 1 in each case, and the very small deficiency of plants with white-margined flowers from the theoretical expectation in these cases may be probably due to the first of the two causes above mentioned. That in the case of pot culture we see always a definite deficiency, may be perhaps due to the fact that pots are generally too dry in summer without special precautions. As is well known through the investigations of several botanists, the formation of anthocyanin in leaves is very much accelerated when leaves live under very dry conditions. Thus, according to Wheldale¹, we see the development of anthocyanin in *Pelargonium* which was insufficiently watered; also Miyoshi² observed

¹ *The Anthocyanin Pigments of Plants*. Cambridge, 1916, p. 24.

² *Journ. Coll. Science, Tôkyô Imp. University*, Vol. xxvii. 1909, pp. 1—5.

that leaves of trees in the East Indies, Ceylon and Java redden during the dry period in the same way as autumnal leaves do in the temperate regions. Again Pellew¹ reports that the amount of pigment in petals of both white and blue plants of *Campanula carpatica* varies according to the moisture condition of the soil, flowers becoming much darker after rain.

In our case it would not therefore be unlikely that owing to the summer drought some anthocyanin would develop in the "hukurin" part and make white-margined flowers look like fully-coloured ones, especially in plants grown in pots.

3. Flower-colour.

As will be seen from Table I there occur no dark-red flowers in yellow-leaved plants. As flowers of this colour are found exclusively on green plants, it might perhaps be concluded that some coupling or repulsion took place between flower- and leaf-colour. But such is not really the case, as will be easily seen from Table V.

TABLE V.

F ₁ plants	Leaf-colour	Results		Totals	Expected		α	δ
		No. of coloured flowering plants	No. of white flowering plants		No. of coloured flowering plants	No. of white flowering plants		
$A \times B (a+b+c)$	Green	158	42	200	150.00	50.00	± 8.00	± 6.124
$B \times A (d+e+f)$	„	136	52	188	141.00	47.00	± 5.00	± 5.937
Totals		294	94	388	291.00	97.00	± 3.00	± 8.592
$A \times B (a+b+c)$	Yellow	54	19	73	54.75	18.25	± 0.75	± 3.700
$B \times A (d+e+f)$	„	59	13	72	54.00	18.00	± 5.00	± 3.674
Totals		113	32	145	108.75	36.25	± 4.25	± 5.241
Gross totals		407	126	533	399.75	133.25	± 7.25	± 9.997

In this Table plants are classified into two groups according to their flower-colour, i.e. those with white and those with coloured flowers. From this we see that the ratio of individuals of these two classes, both in green as well as yellow plants, is 3 : 1, despite the fact above noticed that in the latter there were found no plants with dark-red flowers. It will be readily understood from these considerations that we have here to deal with neither coupling nor repulsion.

In green plants the number of those with red, dark-red and white flowers respectively is in the ratio 2 : 1 : 1, as shewn in Table VI.

¹ *Journal of Genetics*, Vol. vi. pp. 317—339.

TABLE VI.

F_1 plants	Results				Expected			α		δ
	Red	Dark-red	White	Totals	Red	Dark-red	White	$R+D:W$	$R+W:D$	
$A \times B (a+b+c)$	99	59	42	200	100	50	50	± 8.00	± 9.00	± 6.124
$B \times A (d+e+f)$	87	49	52	188	94	47	47	± 5.00	± 2.00	± 5.937
Totals	186	108	94	388	194	97	97	± 3.00	± 11.00	± 8.529

(c) F_3 Generation.

Seeds were obtained from 31 F_2 plants, with which to study the F_3 generation. The following are the results of these studies.

1. *Leaf-colour.*

It will be seen from Table VII that in respect to leaf-colour we have obtained exactly the same results as in F_2 (compare Table II).

TABLE VII.

Pedigree No. of F_2 plants	Results			Expected		α	δ
	Green	Yellow	Totals	Green	Yellow		
7	8	6	14	10.50	3.50	± 2.50	± 1.620
9	36	7	43	32.25	10.75	± 3.75	± 2.889
22	76	26	102	76.50	25.50	± 0.50	± 4.373
31	80	19	99	74.25	24.75	± 5.75	± 4.265
44	39	11	50	37.50	12.50	± 1.50	± 2.810
45	10	3	13	9.75	3.25	± 0.25	± 1.561
11 (a)	12	6	18	13.50	4.50	± 1.50	± 1.836
14 (a)	6	2	8	6.00	2.00	± 0.00	± 1.225
15 (a)	11	5	16	12.00	4.00	± 1.00	± 1.732
15 (b)	12	1	13	9.75	3.25	± 2.25	± 1.561
39 (b)	7	1	8	6.00	2.00	± 1.00	± 1.225
Totals	297	87	384	288.00	96.00	± 9.00	± 8.485

From their behaviour in F_3 it was apparent that 20 of the 31 F_2 plants were homozygous for leaf-colour. Of these 10 were green and 10 were yellow. Table VIII gives the total number of F_3 plants obtained from these 20 homozygous F_2 individuals.

TABLE VIII.

Leaf-colour of F_2 plants	Total number of families of F_2 plants	Leaf-colour of F_3 plants	
		Green	Yellow
Green ...	10	241	0
Yellow ...	10	0	564

2. "Hukurin."

We have got exactly the same results as in F_2 , as shewn in Table IX (compare Table III).

TABLE IX.

Pedigree No. of F_2 plants	Results			Expected		σ	δ
	White margined	Fully coloured	Totals	White margined	Fully coloured		
14	43	21	64	48.00	16.00	± 5.00	± 2.289
16	37	11	48	36.00	12.00	± 1.000	± 3.000
19	35	14	49	36.75	12.25	± 1.75	± 3.030
31	76	23	99	74.25	24.75	± 1.75	± 4.308
32	20	7	27	20.25	6.75	± 0.25	± 2.250
38	18	5	23	17.25	5.75	± 0.75	± 2.077
39	32	11	43	32.25	10.75	± 0.25	± 2.839
55	24	13	37	27.75	9.25	± 3.75	± 2.634
11 (a)	11	3	14	10.50	3.50	± 0.50	± 1.620
15 (b)	10	2	12	9.00	3.00	± 1.00	± 1.500
Totals	306	110	416	312.00	104.00	± 6.00	± 8.832

From the above Table we see that the ratio of plants with white-margined and with fully-coloured flowers is 3:1, and in this case, when we compare the ratios of the number of these two kinds of plants in the field- as well as in the pot-cultures to each other we see also in the latter case a certain deficiency of plants of white-margined flowers.

We have got 4 families of plants which contain the "hukurin" factor in homozygous condition, and 9 families where it is entirely absent, as shewn in Table X.

TABLE X.

	Total number of families in F_2	White- margined	Fully- coloured
White-margined	...	4	109
Fully-coloured	...	9	0
			301

3. Flower-colour.

It would be *a priori* easily seen from the results in F_2 that all F_2 plants with white flowers will produce in F_3 again those with white ones. Though I could not obtain many seeds from plants of these families the results shewn in Table XI will fully confirm this expectation.

We could find no families of plants which breed true constantly to dark-red flowers.

TABLE XI.

	Pedigree No. of F_2 plants	Leaf- colour of F_2 plants	Flower-colour of F_3 plants		
			Dark-red	Red	White
α	3	green	0	0	23
	10	„	0	0	10
	26	„	0	0	12
β	56	yellow	0	0	56
γ	7	{ green	0	0	8
		{ yellow	0	0	6
	45	{ green	0	0	10
		{ yellow	0	0	3
Totals ...			0	0	128

We have obtained the two families of plants which breed true to yellow leaves and red flowers, as indicated in Table XII.

TABLE XII.

Pedigree No. of F_2 plants	Leaf- colour of F_3 plants	Flower-colour of F_3 plants		
		Dark-red	Red	White
16	yellow	0	48	0
49 (b)	„	0	18	0
Totals ...		0	66	0

The families which segregate into plants with red and those with white flowers are found only among yellow F_2 plants; in F_3 the ratio of red and white is 3 : 1, as shewn in Table XIII.

TABLE XIII.

Pedigree No. of F_2 plants	Leaf- colour of F_3 plants	Results				Expected			α	δ
		Dark- red	Red	White	Totals	Dark- red	Red	White		
17	yellow	0	64	20	84	0	63.00	21.00	± 1.00	± 3.969
18	„	0	77	25	102	0	76.50	25.50	± 0.50	± 4.373
19	„	0	49	13	62	0	46.50	15.50	± 2.50	± 3.410
38	„	0	23	6	29	0	21.75	7.25	± 1.25	± 2.332
39	„	0	43	7	50	0	37.50	12.50	± 5.50	± 3.062
59	„	0	35	20	55	0	41.25	13.75	± 6.25	± 3.211
60	„	0	46	22	68	0	51.00	17.00	± 5.00	± 3.571
Totals		0	337	113	450	0	337.50	112.50	± 0.50	± 9.186

The families of plants which segregate into those with dark-red and those with white flowers are found only among those which remain constantly green in F_3 , and the ratio of dark-red and white is 3 : 1, as shewn in Table XIV.

TABLE XIV.

Pedigree No. of F_2 plants	Leaf- colour of F_2 plants	Results				Expected				α	δ
		Red	Dark red	White	Totals	Red	Dark red	White			
14	green	0	65	14	79	0	59.25	19.75		± 5.75	± 3.849
32	"	0	27	8	35	0	26.25	8.75		± 0.75	± 2.562
55	"	0	37	11	48	0	36.00	12.00		± 1.00	± 3.000
11 (b)	"	0	7	3	10	0	7.50	2.50		± 0.50	± 1.370
23 (a)	"	0	3	2	5	0	3.75	1.25		± 0.75	± 0.967
23 (b)	"	0	8	1	9	0	6.75	2.25		± 1.25	± 1.299
24 (b)	"	0	9	2	11	0	8.25	2.75		± 0.75	± 1.436
Totals		0	156	41	197	0	147.75	49.25		± 8.25	± 6.078

The families of plants which segregate into those with red and those with dark-red flowers are found only among those which segregate into green and yellow plants in F_2 . The results are indicated in Table XV.

TABLE XV.

Pedigree No. of F_2 plants	Leaf- colour of F_2 plants	Results					Grand totals
		Red	Dark-red	White	Totals		
9	green	23	13	0	36	{	43
	yellow	7	0	0	7		
31	green	48	32	0	80	{	99
	yellow	19	0	0	19		
44	green	22	17	0	39	{	50
	yellow	11	0	0	11		
14 (a)	green	4	2	0	6	{	8
	yellow	2	0	0	2		
15 (a)	green	6	5	0	11	{	16
	yellow	5	0	0	5		
15 (b)	green	2	9	0	11	{	12
	yellow	1	0	0	1		
39 (b)	green	3	3	0	6	{	7
	yellow	1	0	0	1		

In the above Table the total number of individuals with red and of those with dark-red flowers in green plants is 108 and 81, respectively, and although this ratio seems to be somewhat different from the expected 2:1, yet the deviation lies within the range of thrice the standard error, because the former is ± 11 and the latter is ± 6.481 .

We had the two families of plants which segregated in the same way as in F_2 , as shewn in Table XVI.

We have no yellow plants with white flowers in No. 11 (a), but this is no doubt due to the small number of experiments. In other families

TABLE XVI.

Pedigree No. of F_2 plants	Leaf- colour	Results			Expected			α	δ
		No. of coloured flowering plants	No. of white flowering plants	Totals	No. of coloured flowering plants	No. of white flowering plants			
22	green	54	21	75	56.25	18.75		± 2.25	± 3.750
11 (a)	„	8	4	12	9.00	3.00		± 1.00	± 1.500
Totals		62	25	87	65.25	21.75		± 3.25	± 4.039
22	yellow	18	8	26	19.50	6.50		± 1.50	± 2.208
11 (a)	„	6	0	6	4.50	4.50		± 1.50	± 1.060
Totals		24	8	32	24.00	24.00		± 0	± 2.450
Gross totals		86	33	119	89.25	89.25		± 3.25	± 4.724

we see that the ratio of individuals with coloured and white flowers is 3 : 1.

In the above Table, if we classify flower-colour of green plants into *red* and *dark-red*, we see that their ratio is 2 : 1, as was the case in F_2 (see Table VI). This is shewn in Table XVII.

TABLE XVII.

Pedigree No. of F_2 plants	Results			Expected		α	δ
	Red	Dark-red	Totals	Red	Dark-red		
22	35	19	54	36.00	18.00	± 1.00	± 3.697
11 (a)	6	2	8	5.33	2.67	± 0.67	± 1.334
Totals	41	21	62	41.33	20.67	± 0.33	± 3.712

(d) F_4 Generation.

Seeds were obtained from 43 F_3 plants. The results in F_4 are shewn below.

1. *Leaf-colour.*

Only the families of plants which segregated into green and yellow plants are shewn in the following Table.

TABLE XVIII.

Pedigree No. of F_3 plants	Results			Expected		α	δ
	Green	Yellow	Totals	Green	Yellow		
9—23	74	21	95	71.25	23.75	± 2.75	± 4.221
22— 1	78	30	108	81.00	27.00	± 3.00	± 4.500
22— 4	51	16	67	50.25	16.75	± 0.75	± 3.544
31— 1	43	15	58	43.50	14.50	± 0.50	± 3.298
31—10	39	9	48	36.00	12.00	± 3.00	± 3.000
44— 2	79	15	94	70.50	23.50	± 8.50	± 4.198
44— 6	34	10	44	33.00	11.00	± 1.00	± 2.693
Totals	398	116	514	385.50	128.50	± 12.50	± 9.817

In the above Table the deviation of the total number ($= \pm 12.50$) is somewhat larger than the standard error ($= \pm 9.817$), but the difference between them is not very large. Furthermore, if we examine each family separately we see that only in No. 44—2 is the deviation larger than the standard error but even here not larger than twice the latter, so that the results in this case are similar to those gained in F_2 and F_3 (see Tables II and VII).

2. "Hukurin."

Seeds were obtained from 17 F_3 plants with white-margined flowers and we had in F_4 8 families of plants which breed true to the "hukurin" condition. The results from 9 families of plants which exhibited the segregation are shewn in Table XIX.

TABLE XIX.

Pedigree No. of F_4 plants	Results			Expected		α	δ
	White- margined	Fully coloured	Totals	White- margined	Fully- coloured		
14— 2	55	26	81	60.75	20.25	± 5.75	± 3.897
14— 9	74	22	96	72.00	24.00	± 2.00	± 4.243
31—10	35	13	48	36.00	12.00	± 1.00	± 3.000
32— 7	29	6	35	26.25	8.75	± 2.75	± 2.562
38— 2	21	9	30	22.50	7.50	± 1.50	± 2.535
39— 1	69	29	98	73.50	24.50	± 4.50	± 3.824
39— 3	26	11	37	27.75	9.25	± 1.75	± 2.633
39— 7	85	33	118	88.50	29.50	± 3.50	± 4.704
55— 4	34	14	48	36.00	12.00	± 2.00	± 3.000
Totals	428	163	591	443.25	147.75	± 15.25	± 10.527

From these results we see that they are in perfect agreement with those obtained in F_3 (see Table IX). As the number of plants with white-margined flowers was relatively smaller in pot- than in field-culture, we find always some deficiency of plants with white-margined flowers under the expected number.

3. Flower-colour.

The results are shewn in Table XX, (a)—(h).

The segregation shewn in (a) is similar to that occurring in F_2 ; that shewn in (b) and in (c) is, respectively, similar to that shewn in Tables XV and XIV. The segregation shewn in (d) was not observed in F_3 . The segregation shewn in (e), (f), (g) and (h) is similar to that in Table XI (α), Table XIII, Table XII, and Table XI (β), respectively.

TABLE XX.

	Pedigree No. of F_2 plants	Leaf-colour	Red	Dark-red	White	Totals	Grand totals
(a)	22—4	{ green	23	11	17	51	67
		{ yellow	13	0	3	16	
(b)	9—23	{ green	51	23	0	74	95
		{ yellow	21	0	0	21	
	22—1	{ green	51	27	0	78	108
		{ yellow	30	0	0	30	
	31—1	{ green	29	14	0	43	58
		{ yellow	15	0	0	15	
	31—10	{ green	22	17	0	39	48
		{ yellow	9	0	0	9	
	44—2	{ green	54	25	0	79	94
		{ yellow	15	0	0	15	
(c)	Totals	{ green	207	106	0	313	403
		{ yellow	90	0	0	90	
	44—6	{ green	0	34	0	34	44
		{ yellow	0	10	0	10	
	14—2	green	0	81	25	106	
	22—11	„	0	18	6	24	
	32—7	„	0	35	17	52	
	55—4	„	0	48	9	57	
	Totals	...	0	182	57	239	
	Totals	...	0	182	57	239	
(d)	9—4	green	0	35	0	35	
	14—19	„	0	96	0	96	
	31—9	„	0	18	0	18	
	32—6	„	0	26	0	26	
	44—3	„	0	35	0	35	
	Totals	...	0	210	0	210	
(e)	10—1	green	0	0	5	5	
	10—5	„	0	0	5	5	
	23—1	„	0	0	11	11	
	32—5	„	0	0	51	51	
	45—1	„	0	0	10	10	
	Totals	...	0	0	82	82	
(f)	22—3	yellow	57	0	20	77	
	38—1	„	24	0	8	32	
	39—1	„	98	0	40	138	
	39—2	„	39	0	16	55	
	39—3	„	37	0	13	50	
	59—12	„	24	0	6	30	
	60—1	„	63	0	24	87	
	Totals	...	342	0	127	469	

TABLE XX (continued)

Pedigree No. of P_2 plants		Leaf colour	Red	Dark red	White	Totals
(g)	16—4	yellow	86	0	0	86
	16—9	..	31	0	0	31
	17—13	..	31	0	0	31
	17—14	..	34	0	0	34
	17—15	..	33	0	0	33
	31—5	..	30	0	0	30
	38—2	..	30	0	0	30
	38—8	..	27	0	0	27
	39—7	..	118	0	0	118
	44—1	..	28	0	0	28
	44—10	..	86	0	0	86
	59—11	..	29	0	0	29
		Totals	563	0	0	563
(h)	17—7	yellow	0	0	30	30
	56—1	..	0	0	44	44
	59—10	..	0	0	25	25
		Totals	0	0	99	99

We may now consider some points in connection with Table XX

(a) In green plants we have 34 plants with coloured and 17 with white flowers, and the ratio may be taken as 3:1, the deviation and the standard error being ± 4.250 and ± 3.092 , respectively. Of the above 34 plants with coloured flowers 23 individuals have red and 11 dark-red ones, thus their ratio is 2:1, the deviation and the standard error being ± 0.333 and ± 2.450 , respectively.

In yellow plants we have 13 plants with red and 3 with white flowers, and the ratio may be taken as 3:1, the deviation and the standard error being ± 1.000 and ± 1.732 , respectively.

(b) In green plants, except No. 44—6, we have 207 plants with red and 106 with dark-red flowers, thus their ratio is 2:1, the deviation and the standard error being ± 1.667 and ± 8.340 , respectively. In No. 44—6 I could find, in spite of careful observations, no green plants with red flowers, but here some yellow plants with dark-red flowers made their appearance, which were never found otherwise. This peculiar case will be the subject of my future paper.

(c) We have 182 plants with dark-red and 57 with white flowers, thus their ratio is 3:1, the deviation and the standard error being ± 2.750 and ± 6.694 , respectively.

(d) All breed true to dark-red flowers.

(e) All breed true to white flowers.

(f) We have 342 plants with red and 127 with white flowers, thus their ratio is 3:1, the deviation and the standard error being ± 9.750 and ± 9.377 , respectively.

(g) All breed true to red flowers.

(h) All breed true to white flowers.

As will be seen from above, there are some cases where the deviation is larger than the standard error, but these differences are not very large, and it may be safely concluded that the results of all these experiments are in accordance with expectation. Furthermore, from the results of F_3 and F_4 we may deduce the following facts:

1. No homozygous green plants with red flowers were found.
2. In the offspring derived from green plants with red flowers leaf-colour always segregates into green and yellow, while the segregation of flower-colour is either exactly similar to that in F_2 , or different from it, in so far as no white flowers are produced.

(e) *Back-crossing and F_2 .*

In 1916 the back-crossing of one F_1 plant ($= A \times B$) by both of the two parents was done.

The results of $F_1 \times A$ are indicated in Table XXI.

TABLE XXI.

Leaf-colour		Red	Dark-red	White	Totals
Green	...	33	0	48	81
Yellow	...	45	0	38	83
Totals ...		78	0	86	164

In this case the flower was either white or magenta as in F_1 , and all coloured flowers were white-margined.

From the results in F_2 it may be *a priori* expected that the ratios of green and yellow plants, and that of red and white flowers, are 1:1 respectively. Indeed we have obtained 81 green and 83 yellow plants, thus our expectation was so perfectly fulfilled that no further comment is necessary. The ratio of plants with red and white flowers is equally 3:1, the deviation and the standard error being ± 4.000 and ± 6.043 , respectively.

The results of back-cross $F_1 \times B$ are shewn in Table XXII.

In this case we should expect from the results of F_2 that leaf-colour would remain constantly green, that the ratio of plants with white-margined and fully-coloured flowers would be 1:1, and finally that the

TABLE XXII.

Green	Red	White margined	25	46
		Fully coloured	21	
	Dark red	White-margined	10	35
		Fully coloured	25	
Yellow	White	...	0	0
	

ratio of plants with red and dark red flowers would be also 1 : 1. Let us now examine Table XXII to see whether or not our expectation is fulfilled. Firstly, all plants are green. Secondly, there are 35 plants with white-margined and 46 fully-coloured flowers, thus their ratio is 1 : 1, the deviation and the standard error being ± 5.500 and ± 4.500 , respectively. Again, there are 46 plants with red and 35 plants with dark-red flowers, the deviation and the standard error being equal to those of the latter case, respectively. Thus we see that in every case the deviation is larger than the standard error, but the differences between them are not large, so that it would not be unreasonable to consider that we see in both cases segregation in the ratio 1 : 1.

The F_1 plant used in the back crosses just above mentioned was self-fertilised; and the results of the examination of the F_2 generation thus obtained, consisting of 651 individuals in all, have fully confirmed those shewn in Table I.

DISCUSSION OF RESULTS.

It will be readily seen from all the experiments above mentioned that the hereditary behaviour of leaf-colour is in exact accordance with that obtained by Takezaki (p. 61).

The results on the "hukurin" are also the same, at least in some cases, as those reported by him, and in such cases the presence of a factor for producing the "hukurin" part has been duly proven.

If we simply classify plants into those which can produce anthocyanin on the corolla, at least partially, and those which cannot, their ratio in F_2 , F_3 , etc., is 3 : 1. Now since, for the formation of anthocyanin, at least two factors are necessary, we may denote them by **C** and **R**, respectively. Then the dark-red colour is to be represented by **CCRR**. The white colour, as we may infer from the results of experiments, should have one of these factors; suppose the latter to be **C**, then

the two parents and the F_1 hybrid are to be represented as follows, respectively :

$$\begin{aligned}\text{parent } A &= \text{CCrr}, \\ \text{,, } B &= \text{CCRR}, \\ F_1 &= \text{CCRr}.\end{aligned}$$

From these considerations it will be quite evident that the ratio of plants with coloured and white flowers is 3 : 1.

I will go now to the consideration of the interrelation existing between the hereditary behaviour of leaf-colour and dark-red flower-colour. Flowers of the latter colour never appear in yellow plants but exclusively in green ones. It was stated before that this constitutes no case of coupling or repulsion (p. 65), and the results of experiments which are now to be described led me to the conclusion that in the presence of a certain factor **D**, *the flower is either dark-red or of some other colour according as the green factor G is in either homo- or heterozygous condition (or altogether absent).*

There are many instances in which the intensity of flower-colour varies according to the homo- or heterozygous condition of the factor concerned in pigmentation. Thus the flower-colour was found to be lighter in heterozygous than in homozygous individuals, for example in *Atropa Belladonna*¹, *Datura Tatula* \times *D. Stramonium*², *Linum usitatissimum*³, and *Antirrhinum majus*⁴. Although our case has not to deal with the intensity of flower-colour, I think that it has to be ranked among the same class of phenomena as those above cited. Similar examples are also found in respect to the pigmentation of other plant organs, as in *Corchorus capsularis*⁵, Egyptian cotton⁶, Indian cotton⁷, and *Phaseolus vulgaris*⁸. Saunders reported an interesting case of the connection between the factors for hoariness of leaves and flower-colour in *Stocks*⁹. Colour is due here to the presence of two factors **C** and **R** in the zygote. In certain strains of Stocks, the hoariness of the leaves has been found to depend also on the presence of two factors **H** and **K**. Between these two pairs of factors there is a certain relationship, viz.

¹ Bateson and Saunders, *Rept. Evol. Com. Roy. Soc.* 1901, pp. 1—160.

² *L.c.*

³ T. Tammes, *Rec. Trav. Bot. Néerl.* Vol. viii. 3, 1911, pp. 201—288.

⁴ R. S. Finlow and I. H. Burkill, *Mem. Depart. Agric. India. Bot.* Vol. iv. 4, pp. 73—92.

⁵ W. L. Balls, *Journ. Agric. Sci.* Vol. ii. 1908, pp. 346—379.

⁶ H. de Vries, *Ber. Deut. Bot. Ges.* Vol. xviii. 1900, pp. 83—90.

⁷ H. M. Leake, *Journal of Genetics*, Vol. i. 1911, pp. 205—272.

⁸ G. H. Shull, *Amer. Nat.* Vol. xvii. 1908, pp. 433—451.

⁹ E. R. Saunders, *Proc. Roy. Soc.* Vol. lxxxv. B, 1912, pp. 540—545.

that the hoariness due to **H** and **K** is only manifested when **C** and **R** are both present. Hence an albino (as regards anthocyanin) may contain both **H** and **K**, and may yet be glabrous because it cannot contain at the same time both **C** and **R**. An anthocyanin form, on the other hand, which is glabrous, carries of course **C** and **R**, but can only contain either **H** or **K**, and not both; when it carries **C** and **R**, as well as **H** and **K**, it is hoary and coloured.

The relationship existing between the factor for leaf-colour and that for flower-colour in our *Convolvulus* is very similar to the last mentioned case in Stocks. The fact that the dark-red colour appears exclusively in flowers of green plants will be explained in like manner as in the case of Stocks. If we denote for example the factor for green leaf-colour by **G** and that for dark-red flower-colour by **D**, then the parents would be

$$A = ggdd,$$

$$B = GGDD.$$

The F_1 hybrid is thus **GgDd**, so that it is heterozygous for the factor **G**. We will suppose that **D** can produce red colour but not dark-red, when **G** is either heterozygous or absent in the zygote.

We should have in F_2 the following plants:

Plants	Colour		No. of plants
	of leaf	of flower	
GGDD	green	dark-red	1
GGDd	"	"	2
GGdd	"	white	1
GgDD	"	red	2
GgDd	"	"	4
Ggdd	"	white	2
ggDD	yellow	red	1
ggDd	"	"	2
ggdd	"	white	1
Totals	16

The above zygotes may be arranged as follows:

Green leaf	{	dark-red flower	3
		red "	6
		white "	3
Yellow leaf	{	dark-red flower	0
		red "	3
		white "	1

That the theoretical expectation just mentioned is well fulfilled, may be seen from Table II (p. 63), Table VI (p. 66), and Table V (p. 65).

The offspring derived from these F_2 plants were studied in order to ascertain, whether the production of the F_2 plants with the above mentioned genotypic constitutions has been realised.

In the families containing plants which always produce white flowers, Table XI shews that α corresponds to the formula **GGdd**, β to **ggdd** and γ to **Ggdd**.

We could get no family corresponding to the formula **GGDD** in F_3 , though we had some (cf. Table XIV) corresponding to the formula **GGDd**. It will be noticed here that notwithstanding the fact that there should be theoretically one **GGDD** and two **GGDd** in F_2 we had seven **GGDd** and none of **GGDD**, but this may perhaps be merely a matter of chance and without special meaning.

The results in respect to the plants of other genotypic constitutions are as follows :

Table XVI corresponds to GgDd .			
„	XV	„	„ GgDD .
„	XII	„	„ ggDD .
„	XIII	„	„ ggDd .

Thus all results secured in F_3 progenies are fairly well in accordance with the theoretical expectation, except **GGDD**.

Furthermore, let us examine the results in F_4 to see whether or not our expectation is fulfilled. First of all, we have the families corresponding to **GGDD** in Table XX (*d*), and other families are similar to those in F_3 . It will be noticed also here that we have had no single constant family containing green plants with red flowers till we have attained the F_4 generation, and moreover, according to our theoretical expectation it should appear neither in F_2 nor F_3 . This fact alone suffices perhaps to confirm our hypothesis above mentioned that in the presence of the factor **D, G** will produce dark-red colour in its homozygous and red colour in its heterozygous condition.

Next I will pass on to the results of back-crossing. According to our theory the ratio of plants with dark-red and red flowers in $F_1 \times B$ should be 1 : 1, and this was really the case, as will be seen in Table XXII. In $F_1 \times A$ there should be no plant with dark-red flowers, and this is really the fact, as will be seen in Table XXI. Thus again the results of back-crosses are in perfect accordance with our expectation.

Further, I have made various crosses between some of the F_3 individuals to each other, and also between them and either one of the

two original parents. The results of these experiments are shewn in the Table XXIII.

TABLE XXIII.

Crosses attempted	Leaf-colour	Flower-colour				Grand totals
		Red	Dark-red	White	Totals	
(10— 1) × (16— 9)	{ green	50	0	0	50 {	50
	{ yellow	0	0	0	0 }	
(17— 7) × (17—13)	{ green	0	0	0	0 {	64
	{ yellow	64	0	0	64 }	
(22— 3) × (22— 4)	{ green	18	0	3	21 {	51
	{ yellow	27	0	3	30 }	
(22— 3) × (22—11)	{ green	7	0	2	9 {	9
	{ yellow	0	0	0	0 }	
(31— 1) × (31— 5)	{ green	29	0	0	29 {	51
	{ yellow	22	0	0	22 }	
(31—10) × (31— 9)	{ green	24	25	0	49 {	49
	{ yellow	0	0	0	0 }	
(44— 2) × (44—10)	{ green	26	0	0	26 {	70
	{ yellow	44	0	0	44 }	
(55—15) × (38— 1)	{ green	4	0	7	11 {	11
	{ yellow	0	0	0	0 }	
(56— 1) × (38— 1)	{ green	0	0	0	0 {	48
	{ yellow	27	0	21	48 }	
(59—10) × (16— 9)	{ green	0	0	0	0 {	39
	{ yellow	39	0	0	39 }	
<i>A</i> × (9— 4)	{ green	10	0	0	10 {	10
	{ yellow	0	0	0	0 }	
<i>A</i> × (16— 9)	{ green	0	0	0	0 {	9
	{ yellow	9	0	0	9 }	
<i>A</i> × (38— 1)	{ green	0	0	0	0 {	14
	{ yellow	8	0	6	14 }	
(32— 5) × <i>B</i>	{ green	0	11	0	11 {	11
	{ yellow	0	0	0	0 }	

From what was above described about the results of self-fertilisation of F_3 plants it is clear that the genotypic constitutions of F_3 plants and the relative number of various kinds of the offspring as the results of their crosses are as follows:

Let us see whether the results of these experiments and our expectation are in agreement with each other.

(10—1) × (16—9): All the hybrids have the genotypic constitution **GgDd** and should be green-red.

(17—7) × (17—1): All the hybrids have the genotypic constitution **ggDd** and should be yellow-red.

TABLE XXIV.

Crosses attempted	Genotypic constitutions of the parents	Genotypic constitutions of the offspring	Ratio
(10—1) × (16—9)	Ggdd × ggDD	GgDd	1
(17—7) × (17—13)	ggdd × ggDD	ggDd	1
(22—3) × (22—4)	ggDd × GgDd	GgDD, GgDd, Ggdd, ggDD, ggDd, ggdd	1:2:1:1:2:1
(22—3) × (22—11)	ggDd × GGDD	GgDD, GgDd, Ggdd	1:2:1
(31—1) × (31—5)	GgDD × ggDD	GgDD, ggDD	1:1
(31—10) × (31—9)	GgDD × GGDD	GGDD, GgDD	1:1
(44—2) × (44—10)	GgDD × ggDD	GgDD, ggDD	1:1
(55—15) × (38—1)	Ggdd × ggDd	GgDd, Ggdd	1:1
(56—1) × (38—1)	ggdd × ggDd	ggDd, ggdd	1:1
(59—10) × (16—9)	ggdd × ggDD	ggDd	1
A × (9—4)	ggdd × GGDD	GgDd	1
A × (16—9)	ggdd × ggDD	ggDd	1
A × (38—1)	ggdd × ggDd	ggDd, ggdd	1:1
(32—1) × B	Ggdd × GGDD	GGDd	1

(22—3) × (22—4): The genotypic constitution of these hybrids is very various, as will be seen in the above table. If we classify them according to leaf- and flower-colour there should be 3 green-red, 1 green-white, 3 yellow-red, and 1 yellow-white. Now according to this expectation the deviation and the standard error are ± 2.250 and ± 1.985 , respectively, in green plants, and they are ± 4.500 and ± 2.371 , respectively, in yellow ones. Thus the results agree almost entirely with our expectation.

(22—3) × (22—11): The genotypic constitution is here also various, as seen in the above Table. The ratio of red and white should be 3:1, and the experimental result 7:2 shews that our expectation is almost perfectly fulfilled.

(31—1) × (31—5): As the hybrids have the constitution GgDD and ggDD, the ratio of green-red and yellow-red should be 1:1, and the plants really obtained are 27 and 22: thus here also our expectation has been fairly well fulfilled.

(31—10) × (31—9): As the hybrids have the constitution GGDD and GgDD, all plants should be green and the ratio of red and dark-red 1:1, and we have had 25 red and 24 dark-red, the results being thus fairly well in accordance with our expectation.

(44—2) × (44—10): The hybrids have the constitution GgDD and ggDD, so that green-red and yellow-red should be in the ratio 1:1. As, however, the plants of these two kinds are 26 and 44, respectively, their ratio seems to fit somewhat badly with the expectation, but the deviation

and the standard error being ± 9.000 and ± 4.183 , respectively, the results may be said to be in accordance with the expectation.

(55—15) \times (38—1): As the hybrids have the constitution **GgDd** and **Ggdd**, there should be 1 green-red and 1 green-white, and we have 4 green-red and 7 green-white in reality. Though the empirical numbers are very small, the deviation and the standard error are ± 1.500 and ± 1.658 , respectively, and our expectation is fulfilled.

(56—1) \times (38—1): As the hybrids have the constitution **ggDd** and **ggdd** all plants should be yellow, and the ratio of red and white 1 : 1, in fact we have 27 yellow-red and 21 yellow-white.

(59—10) \times (16—9): The hybrids have always the constitution **ggDd**, and we have 39 yellow-red.

A \times (16—4): The hybrids have the constitution **GgDd**, and we have 10 green-red.

A \times (16—9): The hybrids have the constitution **ggDd**, and we have 9 yellow-red.

A \times (38—1): As the hybrids have the constitution **ggDd** and **ggdd**, all plants should be yellow and the ratio of red and white 1 : 1, indeed we have 6 yellow-red and 8 yellow-white.

(32—1) \times B: All hybrids have the constitution **GGDd**, and we have obtained 11 green-dark-red.

When we examine all the above results we find some cases which seem to fit badly with our theoretical expectation, though all these lie within the range allowed by the theory, and such cases are no doubt due to the small number of individuals included in each family. It may be noticed moreover that, on the one hand, all kinds of plants which are theoretically expected to occur in any family were found there to appear; and on the other, in no single family plants of such kinds which should not occur there according to our theoretical expectation were ever found to appear.

SUMMARY.

1. The green colour of leaves is dominant to yellow, and the segregation in F_2 takes place according to the 3 : 1 ratio.
2. The factor producing the "hukurin" is present in the parent with white flowers. This condition is dominant to full colour and in F_2 the segregation occurs according to the 3 : 1 ratio.
3. The results mentioned in 1 and 2 agree with those obtained by Takezaki.

4. If we denote the one parent by **GGDD** and the other by **ggdd**, there exists the interrelation between the factors **G** and **D**, inasmuch as in the presence of **D** the production of the dark-red flower-colour takes place when **G** is present in homozygous condition, and that of the red (magenta or scarlet) colour, when **G** is present in heterozygous condition or altogether absent. The hybrids F_1 (**GgDd**) will thus bear always flowers of red (= magenta) colour.

EXPLANATION OF PLATE II.

Fig. 1. A dark-red flower from the one parent.

Fig. 2. A white flower from the other.

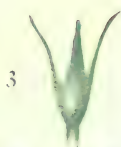
Fig. 3. A magenta flower with "hukurin" from the F_1 plant.

Fig. 4. A scarlet flower from a F_2 plant.

Fig. 5. Leaf and a portion of stem from a green plant.

Fig. 6. Leaf and a portion of stem from a yellow plant.

All figures are from water-colour drawings by Mr N. Midusima.



ON FORMS OF THE HOP (*HUMULUS LUPULUS* L.)
RESISTANT TO MILDEW (*SPHAEROTHECA*
HUMULI (DC.) BURR.): II¹.

By E. S. SALMON,

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IN a previous article¹ attention was called to the fact that certain forms of the Hop (*Humulus Lupulus* L.) are resistant to the attacks of the Hop-mildew (*Sphaerotheca Humuli* (DC.) Burr.). These "immune" plants fall into two groups, (a) certain individual seedlings of the wild hop raised from seed obtained from Vittorio, Italy; (b) the female variety with yellow leaves known as the "golden hop." The present article describes further experiments carried out during 1917 with these and other plants.

Group (a). Of this group 2 seedlings were discovered in 1914, and 7 seedlings in 1916. As already mentioned, the two 1914 seedlings were planted out during the winter 1914—15 in the Experimental Hop-garden at Wye College; the next season one plant (Ref. No. OR 38) proved to be female, the other (Ref. No. OR 39) male. These two plants were sufficiently established by the winter of 1916 to enable "cuts" to be taken from them; 5 were potted up from OR 38 and 2 from OR 39. These potted plants were the ones used in the following experiments (*Expers.* 1 to 5):

Exper. 1. A potted "cut" of OR 39 and a similar potted "cut" of another seedling (Ref. No. Z 54) of the wild hop from Italy were treated as follows: a fully-expanded leaf at the 3rd node from the base of the shoot (which had 7 pairs of leaves) was sprayed with water, using an "atomiser," until numerous small drops had collected on the leaf's upper surface; conidia were then placed on these drops at three similar places on each leaf. The conidia were taken from various "powdery" patches of the mildew occurring on different susceptible hop-plants standing in the greenhouse

¹ See *Journ. Agric. Sci.* Vol. viii, p. 455 (1917).

where the experiment was carried out. In the case of this and of all the experiments described below in order to make the inoculation material as uniform as possible for all the plants in each experiment, the conidia taken from each "powdery" patch were distributed equally as far as possible on all the plants in the process of inoculation. The drops of water were found to have evaporated in a few hours' time.

By the 31st day after inoculation the leaf of Z 54 was infected at the three inoculated places, where clusters of conidiophores occurred. Nine days later these patches had become "powdery" with conidia, and patches of mildew were also present on four other leaves and at four places on the stem. No trace of any infection resulted on OR 39. The two plants stood side by side, and thus exposed to the same chances of inoculation from surrounding mildew-covered plants. In this experiment the unusually prolonged "incubation" period of the mildew was doubtless due to the very abnormal weather conditions of the period (March, 1917) when rapid changes of temperature occurred. Under these conditions the shoots of both plants made scarcely any growth, and the leaves showed a slight injury round their margins which turned brown. Under these adverse conditions of growth the immunity of OR 39 remained unchanged.

Exper. 2. One leaf of a potted "cut" of OR 38, and one leaf of a similar potted "cut" of another seedling (Ref. No. Z 39) of the wild hop from Italy were inoculated as in *Exper. 1*. By the 31st day the leaf of Z 39 bore small patches of mildew with weak clusters of conidiophores at the three inoculated places; the leaf of OR 38 bore similar patches of mildew at two of the three inoculated places. Nine days later the inoculated leaf of Z 39 still bore weak sub-powdery patches at the three places, but the leaf was now beginning to die; on the leaf of OR 38 the weak clusters of conidiophores—scarcely more than "sub-infection"—were still visible at the two places. At the end of the Experiment—58 days from the inoculation—the inoculated leaf of Z 39 had withered up, and all the leaves of the original shoot (which had scarcely elongated under the abnormal weather conditions noted in *Exper. 1*) were brown at their edges; a new basal shoot bore leaves with numerous patches of mildew on them. With regard to OR 38, the original shoot, which had remained checked in growth for some time, had now elongated and was two feet long; there was no trace of any mildew on the inoculated leaf, nor elsewhere on the numerous leaves, although all the leaves were now exposed to frequent inoculations from adjacent mildew-covered plants.

It is clear that the susceptibility shown here by *OR 38* was strictly local or temporary. Various hypotheses may be advanced to account for it: (1) that the conditions of growth temporarily induced some amount of susceptibility; (2) that inoculations where a great number of conidia are used may cause at the place of inoculation a strictly local infection,—as in the cases recorded of “sub-infection”; (3) that the plant *OR 38* is gradually being changed in its “constitution” as the result of cultivation in manured ground; (4) that the conidia used in this Experiment had exceptional powers of infection (which weakened as the mildew grew on the plant, so that the conidia produced there were unable to infect the plant further). The subject is discussed further at page 86.

Exper. 3¹. Two shoots (of equal length and vigour) of two potted “cuts” of *OR 38* and *Z 39* were chosen, and on each shoot 1 leaf (at the 3rd node from the apex) was inoculated at three places². The leaf in each case was just expanded. By the 19th day the inoculated leaf of *Z 39* bore small, densely powdery patches at all the inoculated places, and 4 other leaves on the shoot bore each a powdery patch. No infection resulted on any leaf of *OR 38*.

Exper. 4. Two shoots (of equal length and vigour) of a potted “cut” of *OR 38* and of a seedling plant of the wild hop from Italy—of unknown susceptibility—were chosen, and on each shoot two leaves (at the 3rd node from the apex) were inoculated at three places. On the 12th day the seedling plant was fully infected at all the six places; there were also, by this date, numerous powdery patches on seven other leaves. No infection whatever had occurred on *OR 38*. The same results were recorded on the 21st day.

Exper. 5. The plants used were potted “cuts” of *OR 38*, the var. *neo-mexicanus* (Ref. No. *AA 9*) and a certain seedling (Ref. No. *OC 32*); one leaf (at the 3rd node from the apex) of a vigorous shoot, one foot long, of each plant was inoculated at three places. Each leaf was copiously inoculated with conidia taken from the same sources. On the 7th day the plants *AA 9* and *OC 32* were infected at the three inoculated places on their leaves, where numerous, densely powdery patches occurred; other leaves on both the plants also bore patches of mildew. No trace of infection had resulted on *OR 38*, either on the inoculated

¹ See *The New Phytologist*, Vol. III, p. 110 (1904).

² In this Experiment and in all the following ones, the conditions of growth were normal.

³ In each of these Experiments a different potted “cut” was used.

leaf or elsewhere. On the 16th day¹, however, a leaf (at the 5th node from the apex) of the shoot of *OR 38* bore on its upper surface one tiny, powdery patch of mildew; the inoculated leaf as well as all the other leaves (at the 11 nodes of the stem) of the plant were at this date and subsequently quite free from mildew. There were no signs in this plant of any weakness or abnormal growth, and a close examination of the one leaf bearing the patch of mildew failed to show any difference in its colour or any injury to the epidermal cells which might account for this strictly local susceptibility. The patch of mildew measured 2mm. \times 1mm., and was powdery with conidia. These conidia were removed with a sterilised scalpel, and placed in a film of water on a leaf (at the 2nd node from the apex) of the same shoot of *OR 38*. The other leaf at the same node was similarly inoculated with conidia taken from patches of mildew on another normally-susceptible hop-plant. In neither case did any infection result². The little patch on the leaf of *OR 38* noted above did not increase in size, although it produced a fresh crop of conidiophores, so that 14 days later it was again powdery. The patch then soon began to die away, and 6 days later was dead. After the disappearance of the mycelium, a minute patch of brown, dead epidermal cells became visible, such as is often found at the centre of a flourishing patch of mildew. This plant of *OR 38* grew vigorously throughout the remainder of the season, but although continuously exposed to inoculation by conidia from upwards of a hundred mildew-covered plants surrounding it, no trace of any further infection resulted.

Here, again, as in *Exper. 2* (noted above) there was certainly no general breaking down of the immunity of the plant, but only a strictly local susceptibility. Of the hypotheses advanced above (p. 85) to account for this phenomenon, (2) would appear to be ruled out.

If we summarise the results of the above experiments, we find that the 1 inoculated leaf of *OR 39* resisted infection; of the 5 inoculated leaves of *OR 38*, 4 resisted infection and 1 became feebly infected, while a very small, strictly localised patch of mildew appeared from some unknown source on one uninoculated leaf. Of the 5 leaves inoculated of three other seedlings of the wild hop from Italy all became fully infected, and in the case of each of these seedlings most of the leaves

¹ The date was May 22, 1917.

² A young leaf on this shoot of *OR 38* was pricked with a pin at two places, causing respectively one and three holes, and then inoculated over the wounds with conidia taken from patches of mildew on various hop-plants. No infection resulted. (In other cases susceptibility to the attack of the "wrong" biologic form of a mildew has been induced by this method: see *Annals of Botany*, Vol. xix. p. 125 (1905).)

not inoculated became subsequently infected. The same was also the case with one plant of the var. *neo-mexicanus*, and with one "hybrid" seedling (Ref. No. OC32). It is clear that during the experiments there was no general breaking down of the "immunity" of the two seedlings OR 38 and OR 39, but in the case of OR 38, only a strictly local or temporary susceptibility due to unknown causes. This is confirmed by the general behaviour subsequently of these plants in the greenhouse during the season. Besides the one plant of OR 39 and the four plants of OR 38 used in the above-mentioned Experiments, an additional potted "cut" of each seedling was present in the greenhouse. These seven plants made during the season a strong, "healthy" growth, each producing several stems, 4 to 6 feet high, with large leaves of a dark-green colour. Although the conditions were ideal for the growth and dissemination of the mildew—as was evidenced by the fact that it was only necessary to stand a healthy (susceptible) seedling hop-plant in the greenhouse among the mildewed plants to find it in a few weeks' time more or less smothered with mildew—these seven plants placed under the same conditions for several months showed no trace of infection beyond that temporarily induced in *Expers.* 2 and 5.

The behaviour of the original plants OR 38 and OR 39 may now be noted. These two seedlings proved immune in the greenhouse during the season 1914; during the winter 1914—15 they were planted out in the Experimental Hop-garden at Wye College. In 1916 they flowered, one (OR 38) proving to be female, and the other, male. During the summer of 1916 both plants kept free from mildew¹, although it occurred on all adjacent plants; by October 3rd, however, mildew was observed on both plants. On this date OR 38 showed patches of mildew on several of the leaves and on one hop of a late shoot; while OR 39 showed mildew on one leaf each of two late lateral shoots. In 1917 both plants remained free from mildew¹ until the autumn. In October, however, OR 38 showed a fair amount of mildew (with perithecia) on its hops, chiefly on the peduncles but also on the bracts and bracteoles; OR 39 showed one small patch of mildew on the under-surface of one leaf of a lately-developed lateral shoot.

It is clear, therefore, that both OR 38 and OR 39 when grown in a manured hop-garden produce late in the growing season leaves which are more or less susceptible to mildew, and that OR 38 under these conditions produces "hops" (strobiles) which are decidedly susceptible

¹ No direct inoculations were made on these plants in the hop-garden.

to mildew. Whether the resistance to mildew as shown in 1914 by the original seedling plants of *OR 38* and *OR 39*, and in 1917 by the "cuts" taken from them, will be found to disappear after the plants have been grown for some time in manured ground remains to be seen. It is intended to carry out inoculation experiments with "cuts" taken in successive years.

We will consider now the behaviour in 1917 of the seven seedlings of the wild hop from Italy which showed immunity in 1916.

Exper. 6. One of the above seedlings (plant *a*) and another seedling of unknown susceptibility (plant *b*) of the same parentage and age were inoculated. Each plant was inoculated at three places on 2 leaves (at the 2nd and 3rd node from the apex), the shoot of each plant being of the same length and apparent vigour. By the 18th day plant *b* was infected on one leaf at the three places, where there were large patches of densely clustered conidiophores. By the 27th day the other leaf of plant *b* showed patches of mildew at two of the three places of inoculation. The plant *a* showed no trace of infection.

For the remainder of the growing season this immune seedling *a* together with the six other immune seedlings of 1916 stood in the greenhouse among some hundreds of virulently infected hop-plants, under conditions which ensured a continual inoculation of their leaves and stems with conidia. None of these plants showed a trace of mildew. For two consecutive years, then, these seven seedlings of the wild hop from Italy have proved persistently immune under conditions in which other seedlings of the same parentage and age have proved very susceptible. Three of these seven immune seedlings have now been planted out in the hop-garden.

Group (b). Complete immunity to mildew was shown in 1916 by a form of *H. Lupulus* with yellow leaves obtained under the name of "golden hop" from Messrs Bide and Sons, Nurserymen, Farnham. This plant was further tested in 1917 in the following experiments.

Exper. 7. Two leaves (at the 4th node from the apex) on a plant of the "golden hop" obtained in 1916 from Messrs Bide, and on a two-year-old seedling hop of unknown susceptibility, were inoculated at three places on each leaf. By the 10th day the latter plant bore large densely powdery patches of mildew at the six places of inoculation. No trace of infection was visible at this date, or subsequently, on the "golden hop." This plant, together with three other potted "cuts" of the "golden hop" from the same source, stood in the greenhouse con-

tinuously exposed to infection throughout the season; all the plants remained persistently immune, just as in 1916.

Trial was now made of a yellow-leaved female variety purchased from Messrs Bunyard, Maidstone, in 1912. The plants used were "cuts" taken from established plants in the hop-garden and put into pots in the winter of 1916—17.

Exper. 8. The two plants used were both potted "cuts," one (Ref. No. Z39) was a seedling of the wild hop from Italy, and the other (Ref. No. 341) was the yellow-leaved variety obtained from Messrs Bunyard. On both plants 1 leaf (at the 2nd node from the apex) on shoots of equal length was inoculated at two places with conidia from the same source. Owing to the abnormal weather conditions (see above, p. 84) it was not until the 24th day that any infection was visible on Z39, and then only a few weak conidiophores occurred at one of the places of inoculation; by the 33rd day weak, clustered conidiophores were visible at the two places on the leaf (which was now brown at the edges), and small, vigorous powdery patches occurred on three other leaves and at one place on the stem. No trace of infection resulted on 341, although this plant stood by the side of Z39 throughout the growing season. It was clear that the abnormal weather conditions had no effect upon the immunity of this yellow-leaved plant.

Exper. 9. Two "cuts" in pots of Z39 and 341, with shoots of six nodes and of equal length, were used in this experiment. The two shoots were first "atomised" with water and then inoculated by shaking over them a virulently infected hop-plant, from which the conidia fell in clouds,—with the result that most of the leaves on both shoots became visibly whitened with the mass of conidia. By the 11th day 6 leaves of Z39 were heavily infected, many of the patches of mildew already bearing clustered conidiophores. By the 15th day 7 leaves and parts of the stem were smothered over with powdery patches. No trace of infection occurred on 341, which stood by the side of Z39 throughout the season.

Exper. 10. Shoots of Z39 and of 341 of equal length and vigour were selected, and 2 leaves on each shoot—one partly unfolded leaf at the 2nd node from the apex and one just expanded leaf at the 3rd node—were inoculated at three places on each leaf. By the 13th day the 2 leaves of Z39 were infected at all six places: by the 20th day the 2 inoculated leaves bore densely powdery patches, and 3 other leaves were also mildewed. By the 30th day 14 leaves of Z39 were mildewed.

No trace of infection occurred on 341, which stood by the side of Z39 throughout the season.

Six plants of this "golden hop" (341) from Messrs Bunyard stood exposed to infection in the greenhouse throughout the growing season; all remained persistently immune¹.

The "golden" leaved plants (mentioned above) obtained from Messrs Bide and Bunyard are both female, and agree in the characters of the shape of the leaf and its coloration. A comparison of mature plants to establish the identity of the two has not yet been possible.

In 1917, as in all previous seasons, the 9 plants ("hills") of the "Golden Hop" planted in the hop-garden showed no trace of mildew on leaves or hops throughout the growing season.

A male variety possessing "golden" leaves also exists. In 1910 some plants were sent to me by Messrs Bunyard under the impression that they were the female plant. These were used in the following experiments.

Exper. 11. A young leaf (half expanded) on a shoot, $2\frac{1}{2}$ feet long of a "cut" in a pot was inoculated with conidia from the same source, the three following plants being used: (1) a seedling (Ref. No. OC 32) of the cultivated variety "Bramling"; (2) the Russian variety "Shpaltski"; (3) the ♂ variety with yellow leaves. By the 7th day the leaf on all the plants was equally and virulently infected; the leaf (now fully expanded) being covered over almost continuously with densely clustered conidiophores.

Exper. 12. Young leaves (partly expanded) on shoots of equal length of the Russian variety "Zemshevi" and the ♂ variety with yellow leaves were inoculated. By the 7th day the yellow-leaved plant showed infection at the three places of inoculation; the "Zemshevi" variety was also similarly infected.

Three other "cuts" in pots of the ♂ variety with yellow leaves were placed in the greenhouse and exposed to inoculation by placing virulently infected plants around them. They all became infected. It is clear that this ♂ plant with yellow leaves is susceptible to a normal degree to *S. Humuli*.

¹ An attempt was made to induce susceptibility by injury to the leaf. A young leaf of 341, attached to the stem, was pricked with a pin, 30 holes being made in the half of the lamina on one side of the mid-rib. The injury inflicted did not kill the leaf cells except those immediately surrounding the hole. The whole leaf was inoculated, but no infection resulted.

The facts as regards the origin and correct name of these yellow-leaved or "golden" forms are hard to discover, owing partly to the impossibility of correspondence with Germany. A certain amount of information is being collected, which it is hoped to publish later.

SUMMARY.

1. Individual seedlings of the wild hop (*Humulus Lupulus* L.) when grown in a greenhouse may be immune as regards leaf and stem to the attacks of the mildew *Sphaerotheca Humuli* (DC.) Burr. This immunity has been shown by the same seedlings throughout the growing season for two consecutive years.

2. Such immune seedlings when planted out in the hop-garden may show susceptibility late in the growing season as regards the leaf and "hop" (strobile).

3. An immune plant in the greenhouse may show strictly local susceptibility without the general immunity being lost. (Expers 2 and 5.)

4. A yellow-leaved female variety of *H. Lupulus* is immune to *S. Humuli*.

5. A yellow-leaved male variety of *H. Lupulus* is susceptible to *S. Humuli*.

STUDIES IN VARIEGATION. I.

By W. BATESON, M.A., F.R.S.

(With Plates III and IV and One Text-figure.)

THE phenomena of variegation due to absence or deficiency of chlorophyll have for some time been a special object of study at the John Innes Horticultural Institution. The interest of the subject lies in the circumstance that in variegated plants an opportunity is given of witnessing somatic distribution of a character, deficiency of chlorophyll, already known to be in many plants a Mendelian recessive. It is true that up to the present time no direct experimental evidence exists sufficient to prove that the characters, presence and absence of chlorophyll, heterozygously combined together in fertilisation, can actually lead to the production of a variegated zygote; but from the general course of the phenomena of mosaicism, presenting not very rarely two allelomorphic differences in juxtaposition in the same plant, we may assume without much reservation that this interpretation is admissible. Baur¹, indeed, speaking of a blue *Veronica* bearing a white-flowered branch, observed by de Vries, is disposed to refer such cases to original mutation by loss, rather than to somatic segregation of characters in heterozygous combination. Evidently there is at present no means of positively distinguishing the two possibilities, but I incline to regard somatic or vegetative segregation as on the whole the more acceptable account. If this hypothesis be the true one, we have in variegation a visible model or plan of segregation by which the properties of the germ-cells are certainly determined in many instances, and we may at least entertain the possibility that in plants segregation in properties *not* thus producing visible somatic effects may also be similarly determined. The series of examples which will be described in the present and succeeding papers illustrate miscellaneous features in this special kind of segregation. Apart from any question of wider application the phenomena are, I think, of obvious genetic importance.

¹ *Einführung*, &c. p. 218, Note.

PART I. *Reversal in Periclinal Chimaeras.*

Variegated plants having a white subepidermal layer extending over a green core, however fertilised, give exclusively white or albino offspring, which of course die after a short existence. Conversely those having a green skin over a white core give green offspring only. The significance of this observation was first emphasized by Baur. We have seen numerous examples of such behaviour in the course of our work, of which a list will eventually be given.

The general appearance of these chimaeras, as Winkler and Baur have called them, is familiar. It is noticeable that in some of them the thickness of the "skin," whether white or green, may remain with great constancy the same over very large areas of leaf-surfaces. In white-skinned forms which are thus regular (e.g. Holly and Box) the deficiency of chlorophyll affects chiefly the subepidermal layer. In other plants (e.g. *Nicotiana colossea* var. *variegata*) there is continual irregularity, some leaves having only the subepidermal layer white, while in others the underlying layers are similarly affected to varying depths. In most white-skinned plants the edges of the leaves are solid white throughout their whole thickness, so that each leaf has the white marginal band characteristic of "*varietates albo-marginatae*" as they are styled in horticulture. The width of these white edges is sometimes fairly constant, but generally varies considerably.

The condition in which the core is white and the skin green is far less common, and hitherto we have seen none in which the green layer is uniformly one cell thick. Generally the edges are for a considerable breadth solid green, the thickness of the green layer diminishing towards the centre of the leaves where the white core shows through, being sometimes entirely exposed (as in *Coprosma*). Irregular bands of solid green are often prolonged from the margin into the middle of such leaves. Several of the green-skinned chimaeras have the peculiarity that their *stems* are destitute of chlorophyll or nearly so. For example, in the green-skinned form of *Coprosma*, of *Vinca major*, and of one of the Pelargoniums the stems are almost white. In the green-skinned ivy-leaved Pelargonium also chlorophyll is almost entirely absent from the stem, but, owing to a great development of red anthocyanin in the cortex, the stems are a full pink, whereas in the white-skinned form of the same plant the anthocyanin is confined to a thin layer of the cortex in the stem. In connexion with this type of variegation mention should be made of another, somewhat analogous, in which the absence

of chlorophyll is carried a stage further. In this, not only is the stem white, but the petioles and the centres of the leaves and stipules are also throughout their thickness destitute of chlorophyll. Of this condition I know only two perfect examples, a *Hydrangea* and the Pelargonium "Freak of Nature" raised by Messrs Cannell. Details of these plants will be given in a subsequent paper.

The occurrence to which I wish now to call attention is a somatic change such that a sport arises in which the relative positions of the green and white parts are reversed. This phenomenon of complete reversal has now occurred in five distinct plants, *Euonymus japonicus latifolius*, *Coprosma Baueri*, and three Pelargoniums, viz. an ivy-leaved variety, and two of the zonal class, Mme Solleroi and Caroline Schmidt. In none of the examples is any evidence forthcoming as to the cause of the change, nor can any suggestion be offered as to the nature of the disturbance provoking it.

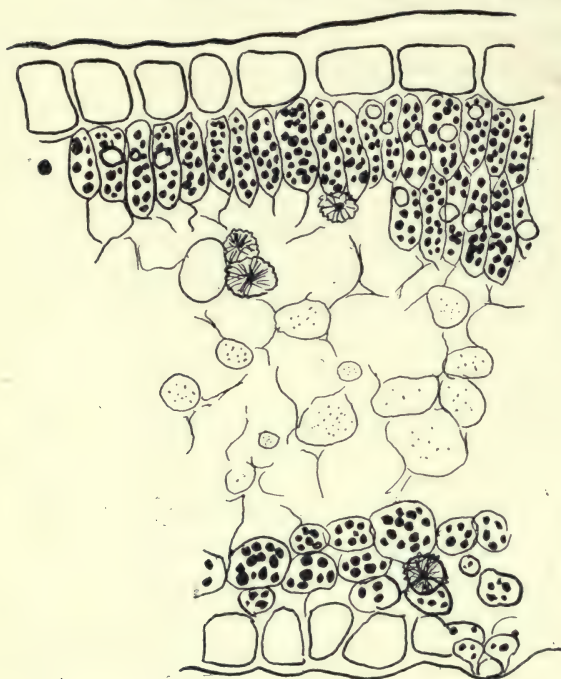
Euonymus japonicus latifolius var. *variegata*.

On the occasion of a visit to Messrs May's nurseries the reversed specimen was noticed among a large batch of well-grown plants of this horticultural variety. The shoot of the green-skinned form¹ was a strong branch arising in a sharply marked area of the stem, well above the level at which the cutting had been divided from the original plant. The growing point of the main stem must, at the point from which the sport arose, have formed simultaneously a white-skinned and a green-skinned segment, and in this latter area a bud had arisen which developed into the green-skinned branch. Neither among the many plants seen at Messrs May's nor among numerous specimens of the variety since examined in various gardens, including several very large plants many feet high, has any similar piece been met with. But wholly white and wholly green areas are formed not uncommonly on the white-skinned variety. If in such an area a bud is included, it gives rise of course to a branch wholly white or wholly green as the case may be. In Fig. 3 a leaf having such a wholly green area is shown. The green-skinned form, on the contrary, though a considerable quantity of it has now been grown, has not produced any substantial variation. In it, as in the white-skinned form, the number of cell-layers forming the "skin" is sometimes greater and sometimes less, but no white areas or white-skinned parts have appeared. The stem in this case is green.

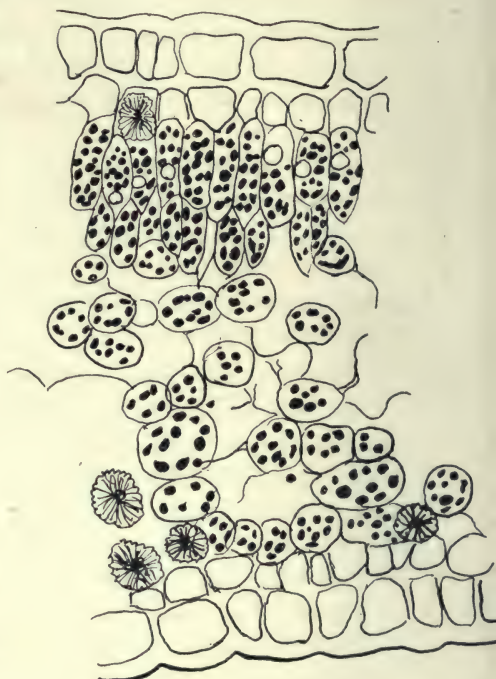
¹ Mr Bintner tells me that this variety is grown in continental nurseries under the name of *Duc d'Anjou*.

The white-skinned form alone has flowered under observation. It failed to set with its own pollen.

The text-figures show in section through the leaves the distribution of chlorophyll in the leaves of the two forms.



Green skin. White core.



White skin. Green core.

Euonymus japonicus latifolius var. *variegata*.

Coprosma Baueri.

The white-skinned var. *variegata* of this New Zealand plant is well known. In 1877 J. Barbier figured in *Rev. Hort. Belg.* III. p. 32 the reversed or green-skinned form which had been lately brought out by Messrs B. S. Williams. To it he gave the name *C. Stocki*. It is also sometimes called var. *picturata*. A few years ago Sir William Lawrence presented to this Institution a cutting of this identical variety which had arisen at Burford as a sport from the ordinary *variegata*. The two forms are shown in Figs. 6 and 7. The distribution of the green in the green-skinned form is approximately the converse of the distribution of the white in *variegata*. Its stems however are white. The green-skinned plant has, with us, produced some wholly green shoots.

Pelargoniums.

In *Pelargonium Mme Solleroi* (Fig. 10) the reversal has occurred on our plants several times. Once a whole branch of green-skinned leaves was formed, for the most part as in Fig. 12, but amongst them a leaf appeared having the whole of one side green as shown in Fig. 14. On another plant of *Mme Solleroi* a shoot appeared bearing many leaves which were wholly white, but the leaf standing lowest on the shoot, viz. the first leaf from the stem, had the structure shown in Fig. 13, half being white and the other half green over white. In individual leaves patches of reversal have been formed as in Fig. 11. Such green-skinned patches include, I believe, always some part of the leaf-margin, and on their internal boundary they are delimited from the white-over-green parts by a white band indicating that in the area in which the two kinds of arrangement abut on each other, the deficiency of chlorophyll extends below the sub-epidermal layer (compare Fig. 16).

It is a peculiarity of *Mme Solleroi* that, so far as I have observed, no flowers are formed on the white-skinned parts, but the green-skinned branch produced a truss of pink flowers. These flowers however were ill-formed¹ and destitute of pollen. The pistils were, I believe, also deformed, but by inadvertence no note of their condition was made.

On a large pink-flowered ivy-leaved *Pelargonium* reversal has also occurred sporadically. Most often the reversal is confined to a part of a leaf, usually *the whole of one side* (as in Fig. 9), but more than one whole branch of the reversed kind has independently appeared. Flowers on the white-skinned parts are fertile, producing (as such plants habitually do) long white carpels in the fertilised fruits, but the green-skinned form has not yet flowered.

The white-skinned *Pelargonium Caroline Schmidt* very often produces wholly green sports. We have here had also several individual leaves on this variety as shown in Fig. 16, composed of a mosaic of the typical and reversed kinds, but hitherto no reversed shoot.

The phenomenon of reversal is evidently rare and exceptional. No example other than those enumerated has yet been seen among the many white-skinned plants grown here or examined elsewhere. We

¹ Note. The variegated *Pelargonium* "Freak of Nature" mentioned above (stem and centres of foliar organs white; edges of foliar organs green) bears deformed flowers having both male and female organs aborted. But sports occur some wholly green, others wholly white, and the flowers on both these are perfect, ripening seed on self-fertilisation, and producing seedlings respectively wholly green or wholly white. This plant has had one small green-skinned branch which has not yet flowered.

have, for instance, several hundred yards of *Euonymus radicans*, var. *variegata* used as an edging-plant. Wholly green shoots are common on this plant, and wholly white shoots not rare, but no reversal has yet been seen. Among many hedges of white-skinned holly also no reversal was found.

Cases superficially mistakable for reversals are not uncommon in various plants. For example, in the white-skinned *Pelargonium* used by Baur in his observations (of which he kindly gave me a cutting some years ago) leaves like that shown in Fig. 15 occasionally appear. At first sight the condition recalls that of Figs. 11 and 16, but on closer examination this is seen to be due in reality to the formation of small solid green areas associated with irregularity in the number of layers devoid of chlorophyll. In this variety, as in many white-skinned forms the appearance of wholly green areas is not very rare.

Obviously the occurrence of reversal, and of areas wholly green or wholly white, are consequences of some instability arising in the growing point, but there is nothing to indicate the cause of such instabilities. The formation of wholly green areas in white-skinned plants may no doubt be described as a bursting out of the green core, and might be attributed to some greater vigour of the green parts, but these expressions are merely descriptive. Injury may be suggested as a probable cause. White shoots do indeed arise with special frequency round old scars on the boles of white-skinned hollies, but green shoots, which might be expected to burst through are extremely rare, if they occur at all in such places. The suggestion of injury is plainly inapplicable to such cases as the *Pelargoniums* described in this paper.

It would be interesting to ascertain whether the green-skinned forms ever change back again, and the absence of any example of this transformation may be worth noting.

As mentioned in introducing the subject, the consequence of somatic reversal is that the genetic properties of the plant are completely changed. Naturally this fact leads to a surmise similar to that suggested by the behaviour of root-cuttings¹. In the variegated chimaeras we can visually distinguish the properties of the cortex, but is it not probable that similar genetic distinctions may exist which are *not* thus visible? May not the phenomenon of reversal exist in regard to them also, bringing into the cortex, and so into the germ-cycle, properties previously contained only in the deeper layers?

¹ See "Root-Cuttings, Chimaeras, and Sports," *Jour. Gen.* Vol. vi. 1916, p. 75.



Fig. 1.



Fig. 3.



Fig. 2.



Fig. 4.



Fig. 6.



Fig. 7.



Fig. 5.



Fig. 8.



Fig. 9.



Fig. 10.



Fig. 11.



Fig. 12.



Fig. 13.



Fig. 14.



Fig. 15.



Fig. 16.

EXPLANATION OF PLATES III AND IV.

The drawings from which these plates were prepared were made by Mr C. H. OSTERBROCK. The various tones of green depend on the number of layers which are green or white respectively.

Fig. 1. *Euonymus japonicus latifolius*, white-skinned variety, having a green core.

Fig. 2. Reversed form of the same, having a green skin over a white core.

Fig. 3. Leaf from another bush of the same having one side wholly green and the other side white skinned. (Slightly too blue in tone: Fig. 4 correctly gives the colour of the dorsal surfaces.)

Figs. 4 and 5. Back and front of a leaf of the same having an area wholly green appearing next the midrib on one side.

Fig. 6. *Cephus Bauri*, var. *variegata*: the white-skinned form.

Fig. 7. The same; green-skinned form.

Fig. 8. Ivy leaved *Pelargonium* showing the mixture of the two kinds of characters.

Fig. 9. Leaf of the same: one side white-skinned the other side for the most part green-skinned.

Fig. 10. Madame Solleroi, zonal *Pelargonium*: the white-skinned form. (The veins in this figure are too wide. They are correctly represented in the other figures.)

Fig. 11. The same with reversed areas at apex of leaf.

Fig. 12. The same: green-skinned leaf.

Fig. 13. The same: leaf all white on one side, green-skinned over most of the other side.

Fig. 14. The same: leaf half green and half green-skinned.

Fig. 15. Baur's white-skinned zonal: leaf showing irregularities in the number of layers deficient in chlorophyll.

Fig. 16. Zonal Caroline Schmidt: leaf with two separate areas of reversal.

INHERITANCE OF CERTAIN CHARACTERS IN THE COWPEA (*VIOLA SINENSIS*).

By S. C. HARLAND, B.Sc. (LOND.).

Imperial Department of Agriculture for the West Indies.

(With One Text-figure.)

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INTRODUCTION.

THE cowpea is a very suitable plant as a subject for genetic investigation in the Tropics. It is easy of culture, occupies little space, and usually produces enough seed per plant for a progeny row of a hundred or more plants. It comes to maturity in from two to three months, and as it can be sown almost at any time of year it is by no means difficult to study three generations of a cross annually. The investigations described in this paper deal with the inheritance of factors concerned in the production of the following characters:

- I. The colour of the flower.
- II. The pattern of the seed coat.
- III. The colour of the pattern of the seed coat.

I. FLOWER COLOUR.

There are three principal types of flower colour in the cowpea. These may be described briefly as follows:

Dark. Possessing much anthocyanin coloration, producing a flower the prevailing colour of which is a more or less deep reddish violet. Colour is most developed in the region of the throat and on the wings. The keel is usually without colour, but a slight amount of violet streaking may be present.

Pale. Distinguished from the Dark form chiefly in the lesser development of anthocyanin coloration. The standard is almost white but the wings are faintly streaked with violet. The keel is devoid of colour.

White. Anthocyanin colour entirely absent, the flower being pure white except for a faint primrose tinge on the standard in the neighbourhood of the throat.

(1) *Dark by Pale.*

Several reciprocal crosses were made. The flower colour of the first hybrid generation was in all cases indistinguishable from that of the Dark parent. Dark is thus completely dominant over Pale.

The F_2 .

In the F_2 three groups of families were grown. The numerical results are presented in Table I. A survey of them shows that segregation occurs into Dark and Pale. The ratio of Dark to Pale is the simple Mendelian one of 3D to 1P.

The F_3 .

In the F_3 a large number of families were grown. The results are to be found in Tables II, III, and IV. From these results it will be seen that in F_3 some of the Darks bred true, while others segregated into Dark and Pale in the 3—1 ratio. The Pales, with certain exceptions explained below, bred true.

Gametic contamination of the F_3 results through Natural Crossing.

The F_3 families of cross 1 from F_2 parents with Pale flowers all bred true. With the exception of one family, this was also true of the families of cross 2. In cross 5, 71 families from F_2 Pales were grown. 14 of these families contained Darks, but in small numbers. At

the time when the F_1 of crosses 1 and 2 were being studied a careful search failed to reveal the presence of any insect likely to cause cross-fertilisation. During the period when the F_2 of cross 5 was in flower a large black carpenter bee (*Xylocopa* sp.) made its appearance, and often visited the flowers. Being heavy bodied this insect caused the extrusion of the stigmas of all the flowers which it visited, and it seems clear that it is capable of cross-pollinating the plants. The presence of a few Darks in families which should have bred true to Pale, must be ascribed to its activities.

Genetic Relationship of Dark and Pale.

On the evidence so far presented it is permissible to conclude that the two types of cowpea flower, Dark and Pale, constitute an allelomorphic pair.

In order to state definitely that the two types differ by a single factor it must be shown that :

1. A ratio of 3 dominants to 1 recessive exists in F_2 .
2. The ratio of pure to heterozygous dominants is 1 : 2.
3. The recessives all breed true.

The results conform to these conditions, for the ratio of pure to heterozygous dominants as shown by their behaviour in F_3 cultures is 83 : 35, i.e. 2.4 : 1.0, a fairly close approximation to expectation. Some of the families were carried to F_4 and F_5 . The results are of no particular significance, as they merely serve to confirm those obtained in previous examinations.

(2) *Dark by White.*

The F_1 of crosses between Dark and White flowered types was Dark. The results of the F_2 and F_3 are presented in Tables V and VI. A summary of the results is as follows :

In F_2 the parental types reappeared and the ratio of Dark to White was 3 Dark to 1 White. In F_3 three families only were grown. Two of these were from F_2 Darks, and showed segregation into Dark and White in the 3—1 ratio. The remaining family was from a White flowered F_2 plant. It bred true to White.

While the evidence does not permit of complete certainty attaching to the conclusion that Dark and White also form an allelomorphic pair, there can be little doubt that such an interpretation of the results is justifiable.

(3) *Pale by White.*

The White used in this experiment was the same as that used in the series of crosses just described. Owing to a great increase in the number of certain insect pests at the time when the cultures of this cross were being grown, it has only been possible to record the F_1 results. In two crosses the F_1 was of the Dark type.

Interpretation of the Experimental Results.

It has been established most clearly that the two colour types Dark and Pale form an allelomorphic pair. From a less complete series of results it has been concluded that Dark and White also form an allelomorphic pair. The F_1 of Pale by the same White proved to be Dark. These results can be explained by the assumption that flower colour in the cowpea is due to the interaction of two factors:

L. A factor for Pale.

D. A factor which increases the amount of anthocyanin colour in the flower but has no visible effect except in presence of L.

According to this hypothesis the number of possible homozygous types resulting from a combination of these two factors is four, viz.:

LLDD	Dark
LLdd	Pale
llDD	White
lldd	White.

Dark (LLDD) and Pale (LLdd) differ in one factor D. Hence they are allelomorphic to each other. Dark (LLDD) and White (llDD) are similarly related. Pale (LLdd) by White (llDD) will give an F_1 of composition LlDd which is Dark and will give in F_2 a ratio of 9D:3P:4W. One of the Whites will be the double recessive lldd.

II. THE PATTERN OF THE SEED COAT.

Dr W. J. Spillman (1911) has described several types of pattern characteristic of the seed coat of various races of cowpea, and in his paper gives a diagram of those that he worked with. A representation of his diagram is given in Fig. 1.



Fig. 1. Forms of the "eye" or pigment area, in the seeds of the cowpea. (After Spillman.)

He refers to Forms *a*—*c* as ordinary eye or small eye. Forms *e* and *f* are treated as one under the name Holstein, from the colour pattern of a variety having that name. Form *d* is called Large eye, and is considered as the heterozygote between Holstein and Small eye. Form *g* represents a genetically different type of eye. In it the pigmented area surrounds the hilum, but the micropylar end of the area has the margin very indistinct: fine dots of pigment extend over the micropylar end of the seed. Spillman calls this latter type the Watson eye, from a variety characterised by this type of pattern.

He investigated the results of 21 crosses between Small eye and Solid colour. In all cases the F_1 was Solid colour. In the F_2 he obtained the following types: Solid, Watson, Holstein, Large eye, and Small eye. The ratio between them approximated to 9:3:1:2:1.

He was able to formulate four hypotheses to explain his ratios. A summary may be given of the hypothesis which he considers as best adapted to explain his results.

The behaviour of the cross Small eye by Solid colour indicates that these two types differ in two factors which are transmitted independently of each other. Accordingly the Watson pattern may be regarded as due to a factor **W**, and the Holstein pattern as due to a factor **H**. The formulae of the types appearing in F_2 will be:

- | | |
|--------------|--------------|
| 1. Solid | WWHH |
| 2. Watson | WWhh |
| 3. Holstein | wwHH |
| 4. Small eye | wwhh. |

The present writer has investigated in detail the results of three crosses between Small eye and Solid colour. In no case have such simple results as those of Spillman been obtained, but the data secured have provided an indirect confirmation of the latter's work. An account of the results obtained in the present work will now be given.

The Experimental Results.

Cross 1. Black eye) by Ronceval¹.
 Small eye) {Solid colour.

Several crosses were made, and the F_1 was in all cases Solid colour.

The F_2 .

The results are presented in Table VII. The following types appeared: Solid, Watson, Holstein types *e* and *f*, Large eye spotted, and

¹ For description see p. 114.

Small eye. These are the same types as were obtained by Spillman, with the exception that the type Large eye spotted replaces the Large eye. Large eye spotted is identical with the Large eye of Spillman except that spots of pigment are usually found on the body of the seed. It must be admitted that certain types would have been classified as Large eye if a very close examination had not been made. Indeed it happened that several pods had to be examined in some plants before the pigment spots were seen. The type Large eye did not appear in any of the crosses studied by the present writer. Large eye spotted was classed as a Holstein. Actually it showed similar genetic behaviour to Spillman's Large eye, and may be regarded as the heterozygote between Small eye and Holstein types e or f .

The present writer does not wish to cast doubt upon the correctness of Spillman's classification, for it is quite probable that the types with which he worked were of different genetic constitution.

A survey of the F_2 results will render apparent the fact that the Solids and Holsteins are in excess of the expected numbers while there is a deficiency in the Watson and Small eye classes. Is the deviation from expectation of genetic significance? Assuming that the ratio 9:3:3:1 is the correct one, then all the Solids will contain both Watson (**W**) and Holstein (**H**), and it is possible to determine which of these factors is responsible for the deviation above alluded to. The proportions in which **W** and **H** occur in the F_2 families will be found in Table VIII, from which it is clear that the deviation from the 9:3:3:1 ratio of Table VII is due to the fact that a greater number of types with the Holstein factor are produced than are expected. The ratio of **W** to **w** is close to 3—1 but the ratio of **H** to **h** is 4.9 to 1.0. Thus from the F_2 results it seems that in this cross the two factor hypothesis of Spillman does not apply.

The F_3 .

The F_3 results are set forth in Table IX. A summary of them is as follows:

1. Solid may either (*a*) breed true, (*b*) segregate into Solid and Watson (**H** : **h** = 4.8 : 1.0), (*c*) segregate into Solid and Holstein (**W** : **w** = 3.1 : 1.0), (*d*) segregate into Solid, Watson, Holstein and Small eye (**H** : **h** = 7.2 : 1.0 and **W** : **w** = 2.6 : 1.0).
2. Watson segregates into Watson and Small eye (**W** : **w** = 2.6 : 1.0).
3. Holstein segregates into Holstein and Small eye (**H** : **h** = 4.7 : 1.0) or breeds true.
4. Small eye breeds true.

It remains now to consider the results in the light of Spillman's hypothesis that in a cross between Solid and Small eye two factors **W** and **H** are concerned. From the F_2 results of Cross 1 it was seen that the ratio of **H** to **h** was 4.9:1.0 instead of 3:1, and that this deviation led to a corresponding deviation in the ratio between the four types. From the above summary of the F_2 results it is evident that the ratio of **H** to **h** is again very abnormal while the ratio of **W** to **w** is of the normal 3=1 type.

According to Spillman's hypothesis the genotypes appearing in F_2 would be as follows:

Homozygous		Heterozygous	
WWHH	Solid	wwhH	Holstein throwing 3H:1SE
WWhh	Watson	wWhh	Watson throwing 3W:1SE
wwHH	Holstein	wWhH	Solid throwing 9S:3W:3H:1SE
wwhh	Small eye	wWHH	Solid throwing 3S:1H
		WWhh	Solid throwing 3S:1H

Thus of every 9 Solids, 1 should breed true, 4 should be dihybrid, 2 should be monohybrid ($S:H$), and the remaining two should be monohybrid ($S:W$). Compare the actual facts. The number of F_2 families from F_2 Solids was 44. Of these 6 bred true, 18 were dihybrid, 5 were monohybrid ($S:W$), and 15 were monohybrid ($S:H$). The corresponding expectation in each case is 4.9, 19.6, 9.8, and 9.8. The proportion of homozygous and dihybrid families is seen to be well in accordance with expectation, but there are three times as many families segregating into Solid and Holstein as into Solid and Watson. Further if this cross were a simple case of the inheritance of two factors it would be found that the ratio of pure to heterozygous Holsteins would be 1:2. This is not so. Out of 22 families grown from F_2 Holsteins, 12 segregate and 10 are pure—a result which indicates that another hypothesis must be sought to explain these facts.

Imagine that the Solid parent instead of being of the constitution **WWHH** was **WWH₁H₁H₂H₂**, i.e. that it contained two factors each of which could produce the Holstein pattern. And suppose also that each Holstein factor could produce the Solid pattern when in association with the Watson factor. The types appearing in the F_2 of the cross **WWH₁H₁H₂H₂** by **wwh₁h₁h₂h₂** would then be: 27**WH₁H₂** (Solid), 9**WH₁h₂** (Solid), 9**Wh₁H₂** (Solid), 9**wh₁H₂** (Holstein), 3**WH₁h₂** (Watson), 3**wh₁h₂** (Holstein), 3**wh₁H₂** (Holstein) and 1**wh₁h₂** (Small eye).

The ratio between the four types would be 45S:3W:15H:1SE. Of the 45 Solids 7 would breed true, 14 would throw S and H, 8 would throw S and W, and the remaining 16 would throw all four types.

Examining the F_3 families in the light of this hypothesis the matter can be set forth as follows:

(a) *The Solids.*

	Number of families			
	<i>S</i> only	<i>S:W:H:SE</i>	<i>S:W</i>	<i>S:H</i>
Expectation with 1H factor ...	4.9	19.6	9.8	9.8
Expectation with 2H factors ...	6.8	15.6	7.8	13.7
Obtained ...	6	18	5	15

(b) *The Holsteins.*

	Number of families	
	<i>H</i> only	<i>H</i> and <i>SE</i>
Expectation with 1H factor ...	14.7	7.3
Expectation with 2H factors ...	11.7	10.3
Obtained ...	12	10

From these results it is evident that so far as the F_3 families are concerned the hypothesis is well confirmed that two Holstein factors are involved in this cross.

The two H factor hypothesis leads to further important consequences. These are:

(a) 4 Solids out of 45 should give in F_3 a ratio of 15*S*:1*W*.

(b) 4 Holsteins out of 15 should give in F_3 a ratio of 15*H*:1*SE*.

A study of the F_3 families which show segregation into *S* and *W* does not lead to the belief that the 15:1 ratio occurs. The number of families is small. Some of the segregating F_3 Holsteins may possibly show the 15:1 ratio, e.g. 1—10—7 (31:3), and 1—10—16 (16:1) but again the number of plants grown is too few to determine this point with certainty.

(c) Out of the 18 families which segregate into all four types in F_3 , 50 per cent. are expected to show the 9:3:3:1, and 50 per cent. the 45:3:15:1 ratio. With such small numbers it is scarcely possible to get more than an approximate idea of whether both types of ratio occur, but taking the families as they stand we have:

Family No. 1—10—26.

	<i>S</i>	<i>W</i>	<i>H</i>	<i>SE</i>
Obtained ...	40	1	8	1
Calculated ...	35.1	2.3	11.7	0.8

Family No. 1—G—9.

	<i>S</i>	<i>W</i>	<i>H</i>	<i>SE</i>
Obtained ...	66	5	19	1
Calculated ...	63.9	4.3	18.3	1.4

on 45:3:15:1 basis.

These results strongly suggest that the 45:3:15:1 ratio does occur.

In regard to the behaviour of the Watsons it is clear that whether one or two Holstein factors are concerned, two Watsons out of three would be monohybrid and the third would breed true. 10 families were grown from F_2 Watsons. All proved to be monohybrid. The absence of the pure type seems to need further investigation. Small eye behaves in F_2 according to expectation and breeds true.

A further point now needs discussion. On the two H factor hypothesis the ratio of H to h in F_2 should be 15:1, and the ratio between SWH , and SE should be 45:3:15:1. This is not found to be the case. The ratio of 49:10 in F_2 of H to h is far from expectation, and allusion has already been made to the abnormal ratios found in the heterozygous families of the F_1 . If the two H factor hypothesis is correct how can the deviation from the 15:1 ratio be accounted for? Two possible explanations suggest themselves.

(a) The Solid parent may have been heterozygous for one of the two H factors, and in that case some of the F_2 families would show the 9:3:3:1 ratio and others the 45:3:15:1 ratio. The numbers obtained in the F_2 families are too small for any discussion of this point to have much value. As they stand, however, they do not lend any support to this theory.

(b) The ratio of 49 H to 1 H in F_2 may be accounted for by assuming linkage between the two H factors. The F_3 results could not be accounted for by such an assumption. No hypothesis can at present be suggested which will explain in all the results. The question is evidently a complicated one, and the whole experiment is being repeated in the hope of elucidating the various points that have arisen.

The unexpected results which were obtained in Cross 1 in respect of the inheritance of pattern on the seed coat led to the study of two other crosses between Solid and Small eye, viz. Cross 2, Red Solid by Black eye, and Cross 5, Red Solid by Brown eye. The results of these two crosses will now be set forth and discussed.

Cross 2. Red Solid by $\begin{cases} \text{Black eye.} \\ \text{Small eye.} \end{cases}$

The F_1 was Solid. In the F_2 segregation occurred into Solid, Watson, Holstein Saddle (types e and f of Spillman), Holstein Large eye spotted, and Small eye. All the Holsteins were classified together. From the numerical results, which are presented in Table X, it will be seen that the ratio of W to w is close to 3—1 while the ratio of H to h

is 312 : 14, i.e. 22:3 : 1:0. Further, the numbers obtained of each type correspond closely with expectation on a ratio of 45 : 3 : 15 : 1. It seems that the hypothesis that two Holstein factors might be involved in a cross between Solid and Small eye holds good in this case without the peculiar complications found in Cross 1.

The F_3 .

The F_3 results are to be found in Table XI. The chief points worthy of notice are as follows:

1. Solid either bred true, segregated into *S*, *W*, *H*, and *SE*, segregated into *S* and *W*, or segregated into *S* and *H*.

The number of families of each of the above types was well in accordance with the two factor hypothesis.

Type of behaviour	Number of families	
	Obtained	Expected
<i>S</i> only	6	4.4
<i>S</i> : <i>W</i> : <i>H</i> : <i>SE</i>	9	8.9
<i>S</i> : <i>H</i>	8	7.8
<i>S</i> : <i>W</i>	2	3.9

It would seem that in the families which segregate into all four types, occur the two types of ratio 45 : 3 : 15 : 1 and 9 : 3 : 3 : 1. As an example of the latter ratio, may be given family No. 2—4—40, and of the former ratio, family No. 2—11—20.

		<i>S</i>	<i>W</i>	<i>H</i>	<i>SE</i>
2—4—40	Obtained	57	18	32	8
	Expected	63.6	21.5	21.5	7.2 (on 9 : 3 : 3 : 1 basis)
2—11—20	Obtained	53	3	22	1
	Expected	55.5	3.7	18	1.2 (on 45 : 3 : 15 : 1 basis)

The two families which segregate into Solid and Watson are apparently both of the 15 : 1 type.

The Solids which segregated into Solid and Holstein did so in the 3—1 ratio.

2. Watson should have exhibited two kinds of behaviour, (*a*) bred true, (*b*) segregated into Watson and Small eye. Actually all the 5 Watsons segregated into Watson and Small eye in the 3—1 ratio. It is somewhat remarkable that no pure Watsons were isolated. The same point was noticed in regard to the Watsons of Cross 1.

3. Holstein bred true or segregated into Holstein and Small eye. Had a representative group of F_2 Holsteins been taken for the F_3 cultures the ratio of pure to heterozygous Holsteins should have been 7 to 8. Ten Holsteins were purposely selected showing the typical

Holstein pattern—the Saddle form (types *e* and *f* of Spillman), and 5 of the Large eye spotted type. It will be seen that the Saddle form bred true while the Large eye spotted proved to be heterozygous. In segregating families the ratio of *H* to *h* appeared to be of the 15:1 type.

4. No families were obtained showing the behaviour of the Small eye form in F_2 .

To sum up: on the whole, the evidence from this cross is in favour of the view that it is of the nature $W H_1 H_2$ by $w h_1 h_2$.

Cross 3. Red Solid by $\begin{cases} \text{Brown eye,} \\ \text{Small eye.} \end{cases}$

The F_1 of this cross was Solid. In the F_2 segregation occurred into Solid, Watson, Holstein (Saddle and Large eye spotted) and Small eye. The results are presented in Table XII.

The ratio of *W* to *w* was again approximately 3:1, while the ratio of *H* to *h* was 10.6 to 1.0. From a preliminary survey of these figures it seems probable that the cross is of the same type as Cross 2, i.e. $W H_1 H_2$ by $w h_1 h_2$. The ratio between the four types was near to 45:3:15:1. In the F_2 a large number of families were grown from F_1 Holsteins in order to obtain more exact figures as to the number of types which were pure and heterozygous respectively. Broadly speaking the Holsteins were classified into two groups, (a) the Large eye spotted form, and (b) the Saddle form (*e* and *f* of Spillman).

Of 106 Holsteins examined in F_2 52 were of the former and 54 were of the latter type.

The F_3 .

The F_3 results are presented in Table XIII. The main points are as follows:

1. The results obtained conform generally to those of Cross 2.
2. The families from F_2 Solids were 23 in number. The number of families which bred true, segregated into *S*, *W*, *H*, and *SE*, into *S* and *W* and into *S* and *H*, was again in accordance with the two *H* factor hypothesis, as will be seen from the summarized results below.

Type of behaviour	Number of families	
	Obtained	Expected
Pure <i>S</i>	2	3.6
<i>S</i> : <i>W</i> : <i>H</i> : <i>SE</i>	7	3.2
<i>S</i> and <i>W</i>	1	1.1
<i>S</i> and <i>H</i>	10	7.1

3. Holstein Large eye spotted proved to be invariably heterozygous. Some of the Holsteins were obviously of the $15H:1SE$ type, but families of the $3H:1SE$ type—e.g. No. 5—5—20 (52:18) were also present. In many families the number of plants is too small to decide which type of ratio prevails. Two of the families apparently segregated into Large eye spotted and Small eye. In these families classification was difficult and some of the plants were referred first to the Large eye spotted form and then to the Saddle.

4. Holstein Saddle proved to be homozygous. There was great variation in the extent to which the pattern was developed on the seed coat, but it was not possible to effect an accurate classification of the differences. It may be possible to distinguish by external appearance the different Holstein combinations, but this would need a more careful examination than the present writer has been able to give.

It will be noticed that a few of the families threw an occasional Solid. This is probably due to accidental crossing by the carpenter bee, *Xylocopa* sp., and the set of results can scarcely be discarded on this account, though due care should be exercised in weighing the whole mass of evidence.

Since Holstein Large eye spotted is always heterozygous and Holstein Saddle homozygous, it is interesting to go back to the F_2 results and consider them anew. In the F_2 of this cross there were altogether 116 Holsteins. Of these 10 were not classified further. Taking the rest, 52 were Large eye spotted, and 54 were of the Saddle form. On the two H factor theory the ratio of pure to heterozygous Holsteins is 7:8, and the ratio actually obtained 7.6:7.4 (54:52). Thus the evidence from the behaviour of F_2 Holsteins in F_3 is strongly in favour of the two H factor hypothesis.

Summary of Results of Investigation on Pattern of the Seed Coat.

In two crosses involving Red Solid and Small eye it has been shown that three independently inherited factors are concerned. These are:

W—The factor for the Watson pattern.

H₁—A factor for the Holstein pattern.

H₂—A second factor for the Holstein pattern, having the same effect as H₁. A combination of W and either of the Holstein factors produces the Solid type of pattern, while absence of all three factors produces the Small eye pattern.

In another cross of Solid and Small eye peculiar ratios were obtained, though the behaviour of certain of the F_2 heterozygous families indicated that three factors were also concerned.

The existence of two Helstein factors producing the same visible effect, alone or in combination, is analogous to the classic case of the colour of wheat grains studied by Nilsson-Ehle (1908) and by the Howards (1912). They found that the Red colour character in certain types was due to three separate units for red. Nilsson-Ehle also observed that a black kernelled oat variety may possess more than one unit for black, each unit alone being able to produce the typical black colour.

One point must here be emphasized most strongly. The present writer, even if he has established little conclusively, has at least demonstrated the inadvisability of fitting a series of results to the nearest probable ratio, and then ascribing excess of deficiency of certain types to chance or to the small number of plants grown.

The results obtained by Dr Spillman are now easily explained. The Solid forms used by him must have been in constitution $W H_1 h_1$ or $W h_1 H_2$. Either of these would give an F_2 ratio of $9S:3W:3H:1SE$, when crossed with Small eye.

III. THE COLOUR OF THE PATTERN OF THE SEED COAT.

(a) *The factor B.*

One of the parents of Cross I, the Black eye variety, is characterised by the appearance of a dark red or purple coloration on the tip of the young pod. The colour first appears when the pod is two or three days old, and lasts until the pod is nearly ripe, when it is obscured by the drying up of the pod which then takes place.

The red tip of the immature pod is a perfectly distinct character, and is found to occur only in plants which possess black coloration of the testa pattern. It is always accompanied by more or less red colour in the calyx and peduncle.

It has been pointed out by Morgan *et al.* (1915) that a so-called unit character is only the most obvious or most significant product of the postulated factor, and that a single factor may affect a plant or animal in many different ways. An example of a single genetic difference which affects the entire organization of a plant is seen in the peculiar types known as Crinkled Dwarf Rogues which occur occasionally in fields of Sea Island cotton. The present writer (1916) has shown that

the normal Sea Island type behaves as a simple dominant to the Crinkled Dwarf Rogue. The latter type differs from normal Sea Island in respect of almost every morphological character and also in certain physiological characters. The whole plant is much smaller; the leaves have a crinkled and mosaic appearance and have ragged edges. Both buds and bolls are shed much more easily than in Sea Island and there is a general reduction in the size of all parts of the plant.

In the case of the association of the red tip of the young pod with the presence of black in the testa, and of coloration in calyx and peduncle, it will be shown that this combination of characters as a whole is allelomorphic to the absence of such a combination, i.e. to pod-tip devoid of colour, seed devoid of black (in this case the colour of the seed is brown), calyx and peduncle without red colour. This may be explained by assuming that several completely coupled factors are concerned, but it seems more reasonable to suppose that all these effects are manifestations of a single factor, *B*.

The Experimental Results.

Cross 1. Black eye by Rounceval.

Black eye. Black present in seed coat; young pod with red tip, red colour present in calyx and peduncle (*B*).

Rounceval. Seed coat uniformly brown, immature pod, calyx, and peduncle green (*b*).

The F_1 showed complete dominance of *B*.

The F_2 and F_3 results.

In Tables XIV and XV will be found the results of the F_2 and F_3 . Reference to these two Tables will show that the ratio of *B* to *b* in F_2 is 3—1, that in F_3 all the families from F_2 recessives bred true and that of the families from F_2 dominants 18 bred true, while 40 segregated into *B* and *b*, again in the 3—1 ratio. The ratio of pure to heterozygous dominants is very close to the expected 1 : 2.

To sum up: the two sets of characters comprised under the symbols *B* and *b*, are allelomorphic to each other.

(b) The Brown and Red colour of the Pattern of the Seed Coat.

In the cross Brown eye by Red, a study was made of the inheritance of the colour of the seed coat pattern. The extent of the colour varies with the extent to which pattern is developed. Thus in dealing with Small eye types it is often impossible to separate different shades, especially those which are even difficult to classify when the colour is

visible over the whole testa, as in the Solid types. In this particular cross, for example, it is certain that at least three different shades of brown appear in F_2 , but owing to the difficulty of classifying accurately, the browns have all been placed in one class.

Brown-eye can be described as a brown with a faint purple cast. The colour of Red is rather paler than that of the variety Red Ripper which is classed by C. V. Piper (1912) as maroon. Piper states that under the group of pink seeded varieties is a range of colours from vinaceous to brick red. When the peas are aged these colours darken so that they are very difficult to distinguish from maroon. In this paper the colour of the variety Red will be described as red. It must be understood that the results which are here described apply only to this particular cross. It is certain that the relations of the testa colours in cowpeas are far from simple, and the working out of all of them would be a task of no mean magnitude.

The Experimental Results.

The plants of the F_1 showed complete dominance of brown, but it was not possible to tell whether the particular shade of brown of the F_1 was the same as that of the brown eye parent.

The F_2 .

The results of the F_2 are presented in Table XVI. Segregation occurred into (a) browns of various shades, (b) dark maroon, difficult to distinguish from black when quite ripe, (c) a maroon less intense in colour, (d) red of the same shade as the red parent.

The plants were classified into three groups, brown, maroon, and red. The ratio between brown, maroon and red was near to 12:3:1. The ratio of brown and maroon to red was 13:2:1.0.

It may be concluded that two factors are concerned, **M** producing the maroon colour, and **N** producing the brown colour. **N** is dominant over **M** and the types appearing in F_2 will be as follows:

- 9 **NM** Brown.
- 3 **Nm** Brown.
- 3 **nM** Maroon.
- 1 **nm** Red.

The various shades of brown and maroon may perhaps be accounted for by assuming that the heterozygotes are different in appearance from the pure types.

In order to confirm this hypothesis a large number of families were grown in F_2 .

The F₃.

The results of the F_3 will be found in Table XVII. It will be seen that:

1. Brown either breeds true (18 families), segregates into brown and maroon (15 families), segregates into brown and red (7 families), or segregates into brown, maroon and red (15 families).

2. Maroon either breeds true (6 families), or segregates into maroon and red (11 families).

3. Red breeds true (12 families).

So far the results appear to confirm the hypothesis which has been put forward. It remains now to examine the proportions in which the various types occur in the segregating families, and also to see if the number of families following the above types of behaviour is in conformity with the hypothesis.

The families which segregate into brown and maroon should do so in the 3—1 ratio. The ratio obtained 348:107 (3·3:1·0) is fairly close to expectation. The 3—1 ratio should also be obtained in families segregating into brown and red. The actual numbers recorded were 18 red and 65 brown, giving a ratio of 2·9:1·0, which is again close to expectation. The proportion of brown, maroon and red in families splitting into all three types should be in the ratio 12:3:1. The summarized results are placed below.

	Brown	Maroon	Red
Obtained	474	116	63
Expected	489·7	122·4	40·8

Here the results again appear to confirm the hypothesis though the number of reds is somewhat above expectation.

The 3—1 ratio is expected in families which segregate into maroon and red. The numbers obtained were 281 maroon and 119 red, a ratio of 2·4 to 1·0. This deviation from the 3—1 ratio is perhaps due to fluctuation.

(a) The Browns.

Type of behaviour	Number of families	
	Observed	Expected
Brown only ...	18	18·3
Brown and Maroon ...	15	9·2
Brown and Red ...	7	9·2
Brown, Maroon and Red	15	18·3

(b) The Maroons.

Maroon only ...	11	11·3
Maroon and Red ...	6	5·7

It will now be considered whether the number of families following the different types of behaviour is in accordance with theory. A comparison of the observed and expected results is given just above.

The main feature of the above results is the difference in the number of families segregating into brown and maroon and brown and red respectively. These should be equal in number whereas those which follow the former type of behaviour are more than double those which are of the latter type. Since, however, the F_2 results as a whole confirm the two factor hypothesis, it is reasonable to suppose that the deviation is due to fluctuation.

At this point it may be observed that the meaning of the various shades of colour found in F_2 became a little clearer by observation of the F_3 families. The observations may be summarized as follows:

1. The brown which segregated into brown and maroon, or into brown maroon and red, was slightly tinged with purple.
2. The brown which segregated into brown and red was very pale and devoid of purple. There was apparently no difference between $Nm Nm$, and $Nm nm$.
3. The darkest shade of maroon was always homozygous. The intermediate maroon segregated into dark maroon, intermediate maroon and red in the 1:2:1 ratio.

Summary of Investigations on the Inheritance of Pattern Colour.

1. The black pattern colour of the variety Black eye is due to a single factor B , which is dominant to its absence.
2. The factors involved in a cross between brown and red are two in number. These are N —a factor which causes the production of brown, and M —a factor which produces a maroon colour. N is dominant over M . In the absence of both factors the seed coat is red. N and M are independent of each other in inheritance.

Relation of Flower Colour to Pattern of the Seed Coat.

In the crosses between Solid and Small eye, it was noticed that a genetic correlation existed between pattern and flower colour. Thus plants containing the Watson factor were always dark flowered. Plants with no Watson factor, i.e. the Holstein and Small eye types, were pale flowered. It may be concluded that Dark flower and Watson pattern are both manifestations of a single factor.

If the flower is white there is no pattern on the seed coat. In a cross between Black eye and a white seeded type (Para), the F_1 was Black Solid and in F_2 segregation occurred into Black Solid, Black

Watson, Black Holstein, Black eye, Brown Solid, Brown Watson, Brown Holstein, Brown eye, and White. The ratios between these types have not been fully worked out, but it is clear that the factor for Dark flower and Watson pattern, for Holstein, and also for Black, can be carried by an albino type (white flowered and white seeded).

The subject of colour correlation in the cowpea has been discussed by Dr Spillman (1913). His note, however, is short, and does not contain the experimental results on which his conclusions are based. Some of his statements may, however, be discussed. According to him all varieties having white or cream coloured seeds have white flowers and are devoid of anthocyanin in stems and leaves. This is not always true. In the cross Red Ripper by Para, a certain number of types in F_2 have white seeds and are devoid of anthocyanin in stems and leaves but also have a distinct violet tinge on the wings of the flowers.

He further states that the flower colour and the anthocyanin in stems and leaves are dependent on two Mendelian colour factors:

- (a) The general factor for colour in the seed coat.
- (b) The special factor for black which when added to a variety having coffee coloured seeds converts the seed colour to black.

It has been shown in this paper that the factor for black affects the seed coat, the pod tip, the calyx and the peduncle. It does not appear to have anything to do with the flower colour or the factor which causes anthocyanin to appear in stems and leaves, except that it is without visible effect except in presence of such a factor. It is, of course, possible that Spillman was working with a different factor for black, and all that the present writer wishes to point out is that Spillman's conclusions are not of universal application.

SUMMARY.

1. In the preceding pages an account has been given of the mode of inheritance of factors affecting the flower colour, pattern of the seed coat, and colour of the seed coat pattern of *Vigna sinensis*. These factors are:

L. A factor which produces the type of flower colour known as Pale. The factor has visible effect only in types with the Holstein and Small eye patterns. It is very possibly the factor mentioned by Spillman (1913) as the one responsible for the production of anthocyanin in stems and leaves.

D. A factor which has no visible effect except in presence of L, when it converts the Small eye pattern to the Watson pattern and the

Holstein pattern to Solid colour, at the same time changing the flower colour to Dark.

H₁. The factor which converts the Small eye pattern to the Holstein pattern.

H₂. The effect of this factor is similar to that of H₁.

B. The factor for black in the seed coat, which also manifests itself by the production of a red tipped pod, and a calyx and peduncle with more or less anthocyanin pigmentation.

N. The factor for a brown or buff colour of the seed coat pattern.

M. The factor for a dark maroon testa pattern.

2. In one cross between Small eye and Solid colour the behaviour of certain of the F_2 families indicated that both the Holstein factors were involved. The ratio of Holstein to No Holstein in F_2 was, however, widely removed from the expected 15:1, being 4.9:1.0. It is pointed out that linkage between the two Holstein factors would account for the F_2 results but not for those of F_3 . No hypothesis can at present be suggested which will account for both F_2 and F_3 results.

3. The following pairs of factors appear to be inherited independently of each other: L and D, D and H₁, D and H₂, H₁ and H₂, M and N.

4. A short statement is put forward in criticism of certain results obtained by Spillman (1913) in regard to colour-correlation in the cowpea.

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TABLE I.

The F₂ results of crosses between Dark and Pale.

Cross	Crosses involving	Family	Dark	Pale	Ratio Dark to Pale
(1) Black eye (Pale) × Rounceval (Dark)		1— 1	26	12	
		1— 2	22	13	
		1— 3	11	5	
		1— 4	30	7	
		1— 5	51	9	
		1— 6	24	8	
		1— 7	108	42	
		1— 8	38	9	
		1— 9	90	25	
		1—10	20	8	
		1—G	54	19	
	Totals	...	474	157	3·0 : 1·0
(2) Black eye (Pale) × Red (Dark)		2— 3	75	19	
		2— 4	69	21	
		2— 5	13	10	
		2— 6	19	9	
		2—10	32	10	
		2—11	37	12	
		2—12	43	10	
	Totals	...	288	91	3·2 : 1·0
(5) Brown eye (Pale) × Red (Dark)		5—G	77	41	
		5— 1	75	25	
		5— 3	16	8	
		5— 5	46	13	
		5— 8	15	3	
		5— 9	105	29	
		5—11	26	9	
		5—12	41	13	
		5—13	22	4	
	Totals	...	423	145	2·9 : 1·0
	Total all crosses	...	1185	393	3·0 : 1·0
	Calculated...	...	1183·5	394·5	

TABLE II

Results of the F₂ generation. Families from F₁ Darks segregating into Dark and Pale

	Family	Dark	Pale		Family	Dark	Pale
Cross 1.	1-5-3	10	4	Cross 2.	2-3-59	28	7
Black eye	1-5-4	34	11	Black eye	2-3-60	42	13
by	1-7-2	10	6	by Red	2-3-66	41	43
Rouneval	1-7-9	12	4		2-3-83	89	35
	1-7-11	27	12		2-4-40	76	40
	1-7-12	14	2		2-4-43	16	5
	1-7-17	66	27		2-4-48	35	8
	1-7-19	16	2		2-4-51	41	17
	1-7-21	18	4		2-4-78	37	11
	1-7-25	11	3		2-4-83	71	36
	1-7-29	16	5		2-11-19	41	16
	1-7-39	55	26		2-11-20	56	24
	1-7-40	15	8		2-11-36	71	17
	1-7-45	61	23		2-11-37	49	16
	1-8-1	28	10		2-12-17	24	16
	1-8-3	30	12		2-12-18	32	12
	1-8-9	30	9		2-12-27	47	11
	1-8-12	21	8		2-12-31	32	11
	1-9-1	25	6		2-12-37	14	4
	1-9-3	16	7		2-12-47	44	16
	1-9-9	13	3		2-12-49	4	3
	1-9-10	16	12	Totals ...		890	331
	1-9-12	16	1	Calculated		915.8	305.2
	1-9-15	16	3	Ratio of Dark to Pale ...		2.7 :	1.0
	1-9-19	6	7				
	1-9-21	12	1				
	1-9-23	11	2				
	1-9-33	25	15	Cross 5.	5-1-37	17	5
	1-10-1	24	8	Brown eye	5-1-50	17	7
	1-10-2	45	17	by Red	5-1-66	15	4
	1-10-3	15	4		5-6-17	11	4
	1-10-5	52	25		5-8-20	20	2
	1-10-9	31	10		5-11-2	8	10
	1-10-12	22	9		5-11-46	15	4
	1-10-17	22	12		5-11-48	15	6
	1-10-19	9	2		5-G-1	13	7
	1-10-20	11	2		5-G-2	17	3
	1-10-23	9	2		5-G-7	9	4
	1-10-26	12	8		5-G-10	12	4
	1-G-6	71	19		5-G-13	12	7
	1-G-23	28	14		5-G-14a	20	15
	1-9-7	21	10		5-G-14b	6	3
	1-9-28	25	6		5-G-23	23	2
	1-9-96	65	19		5-G-26	18	7
					5-G-55	22	9
Totals ...		1122	400	Totals		270	103
Calculated		1141.5	380.5	Calculated		276.8	93.3
Ratio of Dark to Pale...		2.8 :	1.0	Ratio of Dark to Pale ...		2.6 :	1.0

TABLE III.

Results of F₃ generation. Families from F₂ Darks breeding true to Dark.

	Family	Dark	Pale		Family	Dark	Pale
Cross 1.	1— 7—12	15	0	Cross 2.	2— 3—15	17	0
Black eye	1— 7—29	10	0	Black eye	2— 3—17	60	0
by	1— 7—46	41	0	by Red	2— 3—82	28	0
Rounceval	1— 7—75	41	0		2— 4—24	16	0
	1— 8— 2	15	0		2— 4—41	59	0
	1— 9— 2	6	0		2— 4—44	43	0
	1— 9— 8	20	0		2— 4—54	43	0
	1— 9— 9	6	0		2—12— 3	35	0
	1— 9—10	28	0		2—12—14	19	0
	1— 9—11	10	0		2—12—26	52	0
	1— 9—23	13	0	Total	...	372	0
	1— 9—26	6	0				
	1— 9—96	84	0				
	1—10— 2	41	0				
	1—10—19	8	0				
	1—10—23	9	0				
	1—10—25	32	0				
	1—10—26	49	0				
	1—G --24	81	0				
Total	...	515	0				

	Family	Dark	Pale		Family	Dark	Pale
Cross 5.	5— 8— 55	19	0				
Brown eye	5— 8—106	23	0				
by Red	5—G— 5	23	0				
	5—G— 21	45	0				
	5—G— 54	35	0				
	5—G— 56	14	0				
Total	...	159	0				

TABLE IV.

Results of F₃ generation. Pales breeding true to Pales.

	Family	Dark	Pale		Family	Dark	Pale
Cross 1.	1— 5— 3	0	8	Cross 2.	2— 3— 80	0	48
Black eye	1— 7— 7	0	115	Black eye	2— 3— 81	0	16
by	1— 7—11	0	39	by Red	2— 3— 87	0	36
Rounceval	1— 7—22	0	4		2— 3—103	0	28
	1— 7—39	0	80		2— 4— 42	0	12
	1— 7—47	0	11		2— 4— 53	0	49
	1— 7—73	0	96		2— 4— 62 _a	0	6
	1— 8— 1	0	39		2— 4— 62 _b	1	60
	1— 8—10	0	41		2— 4— 65	0	49
	1— 9— 1	0	31		2— 4— 75	0	19
	1— 9— 3	0	23		2—10— 1	0	14
	1— 9—12	0	17		2—10— 21	0	4
	1— 9—17	0	16		2—10— 27	0	25
	1— 9—20	0	5		2—11— 13	0	26
	1— 9—21	0	13		2—11— 21	0	6
	1— 9—22	0	14		2—11— 42	0	47
	1— 9—24	0	21	Totals	...	1 ¹	445
	1— 9—32	0	42				
	1—10—12	0	31				
	1—10—16	0	17				
	1—10—21	0	86				
	1—G— 6	0	90				
	1—G—23	0	41				
	1—G—65	0	27				
Total	...	0	907				

	Family	Dark	Pale		Family	Dark	Pale
Cross 5.	5— 1— 9	0	19				
Brown eye	5— 1— 10	0	34				
by Red	5— 1— 17	0	11				
	5— 1— 19	0	26				
	5— 1— 28	0	18				
	5— 1— 55	0	8				

¹ Probable vicinist.

TABLE IV—*continued*

	Family			Dark	Pale		Family			Dark	Pale
Cross 5. Brown eye by Red	5	1	79	0	21		5	9	3	0	30
	5	2	6	0	59		5	9	6	0	93
	5	3	15	0	41		5	9	8	0	59
	5	3	16	0	30		5	11	26	0	22
	5	5	1	5	64		5	11	36	0	17
	5	5	12	0	18		5	11	50	1	57
	5	5	16	0	24		5	12	11	0	44
	5	5	20	1	70		5	G	10	3	35
	5	5	36	0	52		5	G	12	0	20
	5	5	51	0	82		5	G	16	0	22
	5	5	45	0	35		5	G	19	0	41
	5	5	53	0	26		5	G	20	0	19
	5	5	54	0	7		5	G	22	0	22
	5	8	4	0	36		5	G	24	0	22
	5	8	18	2	37		5	G	25	0	6
	5	8	23a	0	24		5	G	27	2	14
	5	8	23b	1	33		5	G	31a	0	48
	5	8	31	1	61		5	G	31b	2	28
	5	8	35	0	66		5	G	41	0	54
	5	8	50	1	82		5	G	45	1	23
	5	8	59	2	35		5	G	49	0	24
	5	8	67	0	41		5	G	50	0	13
	5	8	77	0	78		5	G	51	0	15
	5	8	79	0	65		5	G	58	0	16
	5	8	81	0	81		5	G	77	0	20
	5	8	85	0	35		5	G	86	0	23
	5	8	94	3	47		5	G	88	0	6
	5	8	101	0	19		5	G	98	2	25
	5	8	109	0	36		5	G	100	0	70
	5	8	111	0	33		5	G	104	0	6
	5	8	113	0	12		5	G	11	0	30
	5	8	115	0	44						
	5	8	117	0	92	Totals	30 ¹	2626
	5	8	135	3	40						

¹ Probably vicinists.TABLE V. *The F_2 results of crosses between Dark and White.*

Family	Dark	White
8—1	25	8
8—2	24	12
A—1	12	5
A—2	25	8
A—3	25	7
Totals ...	111	40
Calculated	113.3	37.8
Ratio ...	2.8	1.0

TABLE VI. *The F_3 results of crosses between Dark and White.*1. Families from F_2 Darks segregating into Dark and White.

Family	Dark	White
6—1—1	21	8
6—1—2	32	15
Totals ...	53	23
Calculated ...	57	19
Ratio ...	2.3	1.0

2. Family 6—1—3 from a White F_2 threw 45 whites in F_3 .

TABLE VII.

The F₂ generation of the cross Small eye (type c) by Solid colour.

	Family	Solid	Watson	Holstein	Small eye
Cross 1.	1— 1	19	5	9	3
Black eye by Brown	1— 2	13	2	12	0
	1— 3	7	3	2	2
	1— 4	19	6	6	0
	1— 5	36	5	6	2
	1— 6	18	2	5	1
	1— 7	88	13	28	9
	1— 8	31	7	6	3
	1— 9	80	9	20	5
	1—10	16	4	7	1
	1—G	38	12	18	1
Totals ...		365	68	119	27
		326 ¹	109	109	36

¹ Highest expectation on 9 : 3 : 3 : 1 basis.

TABLE VIII.

Showing proportions in which W and H occur in the F₂ of Cross 1.

Family	W	w	Ratio	H	h	Ratio
1— 1	26	12		28	8	
1— 2	22	13		25	2	
1— 3	11	5		9	5	
1— 4	30	7		25	6	
1— 5	51	9		43	11	
1— 6	24	8		23	3	
1— 7	108	42		116	23	
1— 8	38	9		37	10	
1— 9	90	25		100	14	
1—10	20	8		23	5	
1— G	54	19		56	13	
Totals ...	474	157	3.0 : 1.0	485	100	4.9 : 1.0
Calculated	473.3	157.8		488.8	146.3	

TABLE IX.

Analysis of pattern in F₃. Cross 1. Black eye by Rounceval.

For convenience use will be made of the following symbols :

S = Solid, H = Holstein, W = Watson, E = Small eye.

	Family	S	W	H	E
(1) The Solids	1— 7— 9	24	1	9	1
	1— 7—11	25	2	9	3
	1— 7—12	21	5	7	1
	1— 7—29	5	2	1	2
	1— 7—25	17	5	4	1
	1— 7—45	51	10	21	2
	1— 8— 1	28	1	8	2
	1— 8— 3	24	5	10	1
	1— 8—17	17	4	7	0
	1— 9— 3	15	1	7	0
	1— 9—19	3	2	5	0
	1— 9—23	8	3	2	0
	1— 9—27	11	4	21	1
	1—10—19	4	2	2	0

TABLE IX—*continued*

	Family	S	W	H	E
1. The Solids	1-10-20	7	3	0	1
	1-10-26	40	1	8	1
	1-G-6	66	5	19	1
	1-G-23	27	0	11	3
	1-9-14	8	1	—	—
	1-9-22	10	4	—	—
	1-9-25	84	11	—	—
	1-10-27	38	13	—	—
	1-7-2	9	—	6	—
	1-7-19	15	—	2	—
	1-7-21	18	—	4	—
	1-7-40	15	—	7	—
	1-9-9	3	—	3	—
	1-9-12	16	—	1	—
	1-9-15	15	—	3	—
	1-9-21	12	—	1	—
	1-9-96	65	—	19	—
	1-10-1	24	—	8	—
	1-10-2	31	—	10	—
	1-10-3	15	—	4	—
	1-10-5	52	—	25	—
	1-10-17	20	—	3	—
	1-10-23	7	—	2	—
	1-7-45	41	—	—	—
	1-8-10	41	—	—	—
	1-9-8	20	—	—	—
	1-9-17	16	—	—	—
	1-9-24	21	—	—	—
	1-10-25	32	—	—	—
Summary :	S	W	H	E	Ratio
	393	56	151	20	W : w = 449 : 171 = 2·6 : 1·0
	140	29	—	—	H : h = 544 : 76 = 7·2 : 1·0
	317	—	103	—	H : h = 4·8 : 1·0
2. The Watsons	Family	W'H	W'h	w'H	w'h
	1-5-3	—	6	—	2
	1-5-4	—	27	—	9
	1-7-17	—	66	—	26
	1-7-39	—	54	—	26
	1-7-47	—	10	—	1
	1-9-1	—	25	—	6
	1-9-10	—	16	—	12
	1-9-28	—	19	—	4
	Totals	—	223	—	86
Ratio	Calculated	—	231·8	—	77·2
	Ratio	—	2·6	—	1·0
3. The Holsteins	1-7-18	—	—	14	2
	1-7-27	—	—	17	4
	1-7-73	—	—	79	17
	1-8-14	—	—	25	10
	1-9-2	—	—	5	1
	1-9-11	—	—	9	1
	1-9-20	—	—	3	2
	1-10-7	—	—	31	3

*Inheritance in the Cowpea*TABLE IX—*continued.*

	Family	WH	Wh	wh	wh
3. The Holsteins	1—10—16	—	—	16	1
	1—10—18	—	—	14	1
	1—10—21	—	—	69	17
	1—G—24	—	—	66	15
	Totals ...	—	—	348	74
	Calculated ...	—	—	316·5	105·5
	Ratio ...	—	—	4·7	1·0
	1— 7— 6	—	—	16	—
	1— 7—22	—	—	4	—
	1— 7—30	—	—	8	—
	1— 7—35	—	—	18	—
	1— 8— 2	—	—	15	—
	1— 9—32	—	—	42	—
	1—10— 8	—	—	14	—
	1—10—13	—	—	46	—
	1—G—53	—	—	37	—
	1—G—65	—	—	27	—
	Total ...	—	—	227	—
4. Small eye	1— 7— 7	—	—	—	117
	1— 7—46	—	—	—	41
	1—10— 4	—	—	—	37
	Total ...	—	—	—	195

TABLE X.

The F₂ results of the cross Red Solid by Black eye.

Family	Solid	Watson	Holstein	Small eye
2— 3	64	5	18	—
2— 4	63	3	19	—
2— 5	3	—	4	—
2— 6	12	—	6	—
2—10	31	1	9	1
2—11	36	—	11	1
2—12	37	3	9	—
Totals ...	246	12	76	2
	236·3 ¹	15·8	78·8	5·3

¹ Expectation on a 45 : 3 : 15 : 1 basis.

TABLE XI.

The F₃ results of the cross Red Solid by Small eye.

S = Solid, W = Watson, H = Holstein, E = Small eye.

	Family	S	W	H	E
1. The Solids	2— 4—40	57	18	32	8
	2— 4—48a	14	2	5	1
	2— 4—48b	28	6	4	4
	2— 4—83	65	1	35	1
	2—11—19	11	0	5	1
	2—11—20	53	3	22	1
	2—11—37	47	1	15	1
	2—12—17	18	3	12	3
	2—12—47	39	5	15	1

TABLE XI—*continued.*

	Family	S	W	H	I
1. The Solids	2—4—41	57	2	—	—
	2—4—54	42	1	—	—
	2—3—59	26	—	7	—
	2—3—60	42	—	13	—
	2—3—83	83	—	35	—
	2—4—51	40	—	17	—
	2—11—36	70	—	16	—
	2—12—18	30	—	12	—
	2—12—27	46	—	11	—
	2—12—37	13	—	3	—
	2—3—15	24	—	—	—
	2—3—17	47	—	—	—
	2—4—24	15	—	—	—
	2—12—3	35	—	—	—
	2—12—14	13	—	—	—
	2—12—26	41	—	—	—
2. The Watsons	2—3—66	—	34	—	11
	2—3—72	—	1	—	2
	2—4—43	—	6	—	4
	2—12—31	—	23	—	9
	2—12—49	—	5	—	3
Totals		—	69	—	29
Calculated		—	73.5	—	24.5
3. The Holsteins	2—3—80	—	—	44	4
	2—3—81 ^a	—	—	34	1
	2—4—53	—	—	42	6
	2—4—62	—	—	56	3
	2—3—81 ^b	—	—	16	—
	2—3—103	—	—	28	—
	2—4—42	—	—	12	—
	2—4—65	—	—	39	—
	2—4—75	—	—	17	—
	2—10—1	—	—	13	—
	2—10—27	—	—	25	—
	2—11—13	—	—	26	—
	2—11—21	—	—	6	—
	2—11—42	—	—	47	—

S : H
Ratio = 350 : 114
3.1 : 1.0

TABLE XII.

The F₂ results of the cross Red Solid by Brown eye.

Family	Solid	Watson	Holstein	Small eye
5—G	73	3	34	3
5—1	70	3	22	3
5—3	13	2	7	1
5—5	43	3	7	6
5—6	12	3	1	2
5—8	100	5	22	6
5—9	23	3	8	1
5—11	36	4	11	1
5—12	20	1	4	0
Totals		27	116	23
		390.63	25.0	130.2
				8.7

^a Expectation on 45 : 3 : 15 : 1 basis

TABLE XIII.

*The F₃ results of Cross 5. Red Solid by Brown eye.**S = Solid, W = Watson, H = Holstein, E = Small eye.*

	Family	S	W	H	E	
1. The Solids	5— 1— 50	10	7	6	1	
	5— 6— 17	10	1	4	—	
	5—11— 48	12	3	4	2	
	5—G— 1	12	1	7	—	
	5—G— 23	21	2	2	—	
	5—G— 26	17	1	7	—	
	5—G— 55	21	1	9	—	
	5— 8— 55	17	2	—	—	
	5— 8—106	16	7	—	—	
	5—G— 21	42	3 ¹	—	—	
	5—G— 54	32	2 ²	—	—	
	5— 1— 37	17	—	5	—	
	5— 1— 66	15	—	4	—	
	5— 8—120	20	—	2	—	
	5—11— 2	8	—	10	—	
	5—11— 46	15	—	4	—	
	5—G— 2	17	—	3	—	
	5—G— 7	21	—	11	—	
	5—G— 10	29	—	13	—	
	5—G— 13	31	—	16	—	
	5—G— 14	6	—	3	—	
	5—G— 5	51	—	—	—	
	5—G— 56	14	—	—	—	
						<div style="text-align: right;"> <i>S H</i> <i>Ratio 179 : 71</i> <i>2.5 : 1.0</i> </div>

2. The Watsons. No Watsons grown from this cross.

3. The Holsteins. (1) Saddle Holsteins.

	Family	S	W	H ³	E
	5— 1— 10	—	—	34	—
	5— 1— 17	—	—	11	—
	5— 1— 72	—	—	5	—
	5— 1— 79	—	—	21	—
	5— 2— 6	—	—	59	—
	5— 3— 16	—	—	30	—
	5— 5— 15	—	—	41	—
	5— 5— 16	—	—	24	—
	5— 5— 24	—	—	7	—
	5— 8— 10	1	—	35	—
	5— 8— 23	—	—	57	—
	5— 8— 31	1	—	65	—
	5— 8— 35	—	—	66	—
	5— 8— 67	—	—	41	—
	5— 8— 77	—	—	78	—
	5— 8— 85	—	—	38	—
	5— 8—113	—	—	12	—
	5— 8—115	—	—	44	—
	5— 8—135	—	—	40	—

¹ The two forms bred true (15 and 13), in *F*₄.² The two Watsons bred true in *F*₄ (16 and 6 respectively).³ All Saddle,

TABLE XIII—*continued*

Family	S	H	H (2)	E
5-9-6	—	—	93	—
5-9-8	—	—	59	—
5-11-26	—	—	22	—
5-11-36	—	—	17	—
5-12-11	—	—	48	—
5-G-12	—	—	20	—
5-G-19	—	—	41	—
5-G-20	—	—	19	—
5-G-24	—	—	26	—
5-G-25	—	—	6	—
5-G-31	—	—	47	—
5-G-41	—	—	54	—
5-G-45	1	—	44	—
5-G-50	—	—	82	—
5-G-53	—	—	26	—
5-G-58	—	—	16	—
5-G-77	—	—	20	—
5-G-86	—	—	23	—
5-G-88	—	—	6	—
5-G-100	—	—	70	—

(2) The Holsteins of type Large eye with spots. (Type 2.)

Family	S	H	H (2)	H (5)	E
5-1-9	—	—	11	7	1
5-1-19	—	—	12	12	2
5-1-28	—	—	9	5	4
5-1-55	—	—	3	5	0
5-5-1	—	—	32	31	1
5-5-12	—	—	11	7	0
5-5-20	—	—	35	17	18
5-5-21	—	—	21	24	1
5-5-45	—	—	14	20	1
5-5-51	—	—	48	31	8
5-8-4	4	—	13	17	4
5-8-18	—	—	16	19	2
5-8-23	1	—	14	17	2
5-8-59	—	—	16	18	1
5-8-79	—	—	25	33	7
5-8-81	—	—	45	37	5
5-8-94	—	—	17	29	1
5-8-101	—	—	22	23	4
5-8-109	—	—	14	19	2
5-8-117	—	—	30	41	5
5-9-3	—	—	10	10	10
5-11-50	—	—	42	45	9
5-G-16	—	—	32	—	6
5-G-22	—	—	12	—	10
5-G-27	—	—	8	6	1
5-G-31	2	—	14	9	5
5-G-49	—	—	10	12	2
5-G-50	—	—	26	13	4
5-G-51	—	—	8	6	1
5-G-57	—	—	2	2	1
5-G-98	—	—	9	15	1
5-G-104	—	—	2	4	—
5-G-1	—	—	14	16	1

(3) All Saddle

TABLE XIV.

The F₂ generation of the cross Black eye (B) by Rounceval (b).

Family	B	b	Ratio
1— 1	36	4	—
1— 2	26	8	—
1— 3	9	7	—
1— 4	29	8	—
1— 5	45	15	—
1— 6	25	7	—
1— 7	117	33	—
1— 8	39	8	—
1— 9	83	32	—
1—10	21	7	—
1—G	53	19	—
Totals ...	483	148	3.3 : 1.0

TABLE XV.

The F₃ results of the cross Black eye (B) by Rounceval (b).

(1) Families which segregate into B and b.

Family	B	b
1— 5— 4	28	8
1— 7— 2	14	1
1— 7— 6	12	4
1— 7— 9	12	4
1— 7—17	69	23
1— 7—18	12	4
1— 7—19	10	7
1— 7—21	17	5
1— 7—23	20	5
1— 7—25	12	2
1— 7—27	18	3
1— 7—30	5	3
1— 7—35	14	4
1— 7—40	15	7
1— 7—45	69	15
1— 8— 3	31	9
1— 8—14	23	12
1— 8—17	22	6
1— 9—13	13	3
1— 9—14	7	2
1— 9—15	17	1
1— 9—19	7	3
1— 9—25	72	23
1— 9—27	16	6
1— 9—28	17	6
1—10— 1	12	10
1—10— 2	50	11
1—10— 3	18	1
1—10— 4	24	13
1—10— 5	58	19
1—10— 6	12	3
1—10— 7	26	8
1—10— 8	12	2
1—10—13	36	10
1—10—14	7	4
1—10—17	22	6
1—10—18	13	2
1—10—20	8	3
1—10—27	36	15
1—G—53	29	8

Totals ...	915	281
Calculated ...	897	299
Ratio ...	3.2	1.0

(2) Families which breed true to B.

Family	B	b
1— 7—12	15	—
1— 7—29	10	—
1— 7—46	41	—
1— 7—55	41	—
1— 8— 2	15	—
1— 9— 2	6	—
1— 9— 8	20	—
1— 9— 9	16	—
1— 9—11	10	—
1— 9—23	13	—
1— 9—26	6	—
1— 9—96	84	—
1—10— 2	41	—
1—10—19	8	—
1—10—23	9	—
1—10—25	32	—
1—10—26	49	—
1—G—24	81	—

Total ... 497 —

(3) Families which breed true to b.

Family	B	b
1— 5— 3	—	8
1— 7— 7	—	115
1— 7—11	—	39
1— 7—22	—	4
1— 7—39	—	80
1— 7—47	—	11
1— 7—73	—	96
1— 8— 1	—	39
1— 8—10	—	41
1— 9— 1	—	31
1— 9— 3	—	23
1— 9—12	—	17
1— 9—17	—	16
1— 9—20	—	5
1— 9—21	—	13
1— 9—22	—	14
1— 9—24	—	21
1— 9—32	—	42
1—10—12	—	31
1—10—16	—	17
1—10—21	—	86
1—G— 6	—	90
1—G—23	—	41
1—G—65	—	27

Total ... 907

TABLE XVI.

The F₂ results of Cross 5. Brown eye (Brown) by Red (Red).

Family	Brown	Maroon	Red
5—G	75	30	9
5—1	58	15	5
5—2	12	6	3
5—3	17	6	—
5—4	—	—	—
5—5	40	15	3
5—6	11	4	3
5—8	95	25	10
5—9	26	5	3
5—11	40	10	2
5—12	17	6	1
Totals	391	122	39
	441·6 ¹	110·4	36·8

¹ Expectation on 12 : 3 : 1 basis.

TABLE XVII.

The F₃ results of Cross 5. Brown eye (Brown) by Red (Red).

1. The Browns.

Family	Brown	Maroon	Red
5—1—28	18	—	—
5—3—16	30	—	—
5—5—1	64	—	—
5—5—20	70	—	—
5—5—21	46	—	—
5—5—56	14	—	—
5—8—23	57	—	—
5—8—50	82	—	—
5—8—51	82	—	—
5—8—59	35	—	—
5—8—67	41	—	—
5—8—79	65	—	—
5—8—85	35	—	—
5—8—101	49	—	—
5—8—115	44	—	—
5—11—36	17	—	—
5—G—16	38	—	—
5—G—15	44	—	—
5—G—5	22	6	—
5—G—31	25	3	—
5—G—49	18	6	—
5—G—51	12	3	—
5—G—58	13	3	—
5—G—86	19	4	—
5—1—19	19	7	—
5—1—79	16	5	—
5—2—6	42	17	—
5—5—15	30	11	—
5—8—18	28	9	—
5—8—35	51	15	—
5—8—109	25	11	—
5—8—117	13	1	—

1. The Browns.

Family	Brown	Maroon	Red
5—11—48	15	6	—
5—5—36	37	—	15
5—8—94	38	—	9
5—8—135	28	—	12
5—9—3	24	—	6
5—G—19	31	—	10
5—G—77	14	—	6
5—G—13	22	3	3
5—G—23	17	1	7
5—G—25	4	1	1
5—G—31	35	8	5
5—G—41	34	12	8
5—G—54	23	6	5
5—G—98	15	4	6
5—1—9	15	2	2
5—1—55	4	3	1
5—8—31	47	12	6
5—8—77	60	14	4
5—8—81	63	13	5
5—8—111	22	7	4
5—9—6	69	16	8
5—9—8	43	12	4
5—11—26	18	3	1

2. The Maroons.

5—5—12	13	4
5—8—23	23	10
5—11—2	12	6
5—11—50	56	38

TABLE XVII—*continued.*

2. The Maroons.

Family	Brown	Maroon	Red
5—G—12	—	17	3
5—G—21	—	33	12
5—G—22	—	14	8
5—G—26	—	20	5
5—G—53	—	23	3
5—G—100	—	49	21
<hr/>			
5—5—39	—	4	—
5—5—C	—	14	—
5—5—45	—	35	—
5—8—113	—	12	—
5—G—2	—	20	—
5—G—7	—	21	—

3. The Reds.

Family	Brown	Maroon	Red
5—1—17	—	—	11
5—1—37	—	—	16
5—1—50	—	—	24
5—1—66	—	—	19
5—5—16	—	—	24
5—6—17	—	—	15
5—8—10	2 ¹	1 ¹	35
5—8—55	—	—	19
5—8—106	—	—	23
5—8—120	—	—	22
5—11—46	—	—	19
5—12—11	—	—	48
5—G—10	—	—	42 ²

¹ Probably due to vicinism.² And 1 black.

ON THE RELATION BETWEEN NUMBER OF CHROMOSOMES AND NUMBER OF TYPES, IN *LATHYRUS* ESPECIALLY.

By Ö. WINGE.

(With Plate V.)

THE number of simultaneously and independently segregating pairs of factors¹ in the species investigated by genetic experts has, as we know, never yet been found so high as to exceed the haploid chromosome number of the species. Consequently, there is still nothing to subvert the theory that the genes have their morphological equivalent in the chromosomes and that these latter are—or can be—individually dissimilar in a given biotype as regards the genes included.

It is a question of very great theoretic importance, whether the simultaneously segregating pairs of factors, not mutually connected, can ever exceed the number of chromosomes in the species concerned, this being, so to speak, a decisive point as regards the value of the entire section of the study of chromosomes related to the science of genetics. If a biotype could be found to exhibit segregation of but a single pair of factors in excess of the number indicated by the haploid chromosome value, then, properly speaking, the theory as to the value of chromosomes as bearers of the genetic, segregating units would collapse at once. The nice agreement between reduction division and segregation would thus be irrevocably destroyed.

At a first glance, it might seem likely that we should be able, in highly varying species, to segregate without great difficulty a greater number of types than the chromosome number found for the species permits. We can, however, obtain a surprisingly large number of combinations even from a quite small number of chromosomes, and it must also be borne in mind that the theory of agreement between reduction division and Mendelian segregation would by no means be destroyed even if we did succeed in finding a greater number of independently mending pairs of genes (or pairs of gene-complexes) within a Linnaean

¹ By this expression is meant pairs of factors, or groups of pairs, between which the phenomenon of linkage is not found.

species, than corresponds to the haploid chromosome number. Even a species with but a single chromosome in the haplophase, and two in the diplophase, might be allowed to contain an unlimited number of independently segregating genes, as the two chromosomes in the diplophase might very well be genotypically different between individuals within the species. The point is, that according to the theory, there must not be more independently mendling pairs of factors in a given *biotype* (individual or clone) than the chromosome number of the biotype indicates. As soon as two individuals (not to speak of more) not belonging to the same biotype are introduced into the experiment, the possibility of new combinations is considerably increased, unless the two original individuals are homozygotic.

An organism with only one chromosome in the haplophase ($x=1$) will naturally only be able to have two different chromosomes at the outside in the diplophase. Let us call these A and a . Two different types of gamete can then arise, viz. A and a , and these can form three different types of zygote: AA , Aa , and aa , of which two are homozygotic.

With $x=2$, a given biotype can be doubly heterozygotic; i.e. $AaBb$, and four kinds of gametes can be formed, viz. AB , Ab , aB and ab , of which in F_1 it will be possible to obtain nine diploid combinations, $AABB$, $AABb$, $AAbb$, $AaBB$, $AaBb$, $Aabb$, $aaBB$, $aaBb$, and $aabb$, of which four will be homozygotic in both characters.

Where $x=3$, 8 different gametes can be formed, giving 27 different diploid combinations, of which 8 are homozygotic.

In a word: *With a given haploid chromosome number, x , we can by self-fertilisation and segregation of a single individual obtain, theoretically speaking, at the outside 2^x different gamete types and 3^x different diploid biotypes, of which 2^x will be homozygotic in all characters.*

If we are to entertain any hope of controverting the theory of identity between reduction division and segregation, then naturally it will be necessary to work with organisms having a low chromosome number, and capable of self-fertilisation. A plant with only eight chromosomes ($x=8$) will on self-fertilisation be capable of forming 256 different gametes, and will in F_1 segregate 6561 different types.

If the segregation experiments be commenced with more than one biotype, which of course will as a rule be necessary when working with species not capable of self-fertilisation, the question becomes more complicated.

Two individuals with $x=1$ can in the diplophase differ in both

chromosomes, the one diplobiont having the formula $A A_1$, the other $a a_1$. On formation of the gametes, we can then obtain four different types, $A-A_1-a-a_1$; two from each. In F_1 , we have $2 \times 2 = 4$ genotypically different diploid combinations, viz. $Aa-Aa_1-A_1a-A_1a_1$, and these will, on reduction division, again throw off the four gametes $A-A_1-a$ and a_1 . In F_2 and the following offspring generations, we can by free combination of these find 10 genotypically different combinations, viz. $AA-AA_1-Aa-Aa_1-A_1A_1-A_1a-A_1a_1-aa-aa_1-a_1a_1$, of which four are homozygotic; one for each gamete type.

On considering in the same way an organism with two chromosomes in the haplophase ($x=2$), and presuming the segregation experiment to commence with the crossing of two individuals having different genotypic value for all the chromosomes of the diplophase (eight in all in the two individuals) then each individual will be able to form four gametes, or eight different gametes from both. On crossing these forms, we obtain 16 different biotypes in F_1 ; and in F_2 , where the gametes (16 different) can combine altogether freely, there can arise 100 genotypically distinct diploid types, of which 16 will be homozygotic in all characters, i.e. one for each gamete type formed by F_1 .

Where $x=3$, we can by crossing two different individuals easily obtain, theoretically, eight gametes from each, i.e. 16 different in all. In F_1 , 64 types will have arisen, able to form in all an equal number of gametes, and in F_2 , 1000, of which 64 are homozygotic.

Briefly then, *if a segregation experiment be commenced by crossing two individuals of the species to be investigated, then we can in F_1 obtain 4^x and in F_2 and the following generations 10^x genotypically distinct forms, of which 4^x will be homozygotic in all characters, where x indicates the haploid chromosome number of the species.*

If, for instance, we commence by crossing two entirely different individuals, heterozygotic throughout, of a species with eight chromosomes in the haploid phase, then in F_1 , there can arise 65536, and in F_2 a milliard different types.

On extending the analysis so as to include a further number of individuals, as to whose genotypic constitution nothing is previously known, then the number of possible combinations will of course be far higher even than this, as also when "crossing over" takes place. In this last case we cannot reckon the possibilities beforehand.

The advantage of working with self-fertilising organisms is thus entirely evident. It would be even better if we could make our genetic experiments with organisms where the haplophase was a richly developed

and independently living individual, as for instance in the liverworts. This I have already pointed out in a previous work: "The Chromosomes. Their numbers and general importance" (*Comptes rendus des travaux du Laboratoire de Carlsberg*, Vol. XIII, Copenhagen, 1917). A species such as *Marchantia polymorpha*, with eight chromosomes, would thus only form, at the outside, 256 types on crossing between two individuals; and by taking an individual of a monoecious species, self-fertilised, then *eo ipso* we should obtain but one type—a pure line—if we may use this term also for cultures of haplobionts.

Lathyrus odoratus is one of the plants with which we may hope sooner or later to elucidate the important question as to agreement of reduction division with Mendelian segregation, as students of genetics have for a long time back been occupied with the genotypic features of this species.

Acting upon a suggestion from Prof. R. C. Punnett, of Cambridge, concerning the number of chromosomes in *Lathyrus odoratus*, I proceeded, in the summer of 1918, to fix and examine material of the species in question. In a written communication to me, Prof. Punnett stated that Mr R. P. Gregory had informed him that there were doubtless seven or eight chromosomes in the haplophase. Otherwise, as far as I am aware, nothing is stated in extant literature as to this; no mention is made as to any species of *Lathyrus* either by Tischler in his excellent "Chromosomenzahl, -Form und -Individualität im Pflanzenreiche" (*Progr. Rei Bot.*, Vol. v, 1915, p. 164) or by Ishikawa in his likewise very comprehensive work, "A list of the number of chromosomes" (*The Bot. Magazine*, Tokyo, Vol. xxx, 1916, p. 404).

On two occasions, in the early spring and early summer of 1918, Prof. Punnett sent me, from his cultures at Cambridge, freshly fixed material of young flowers of *L. odoratus*. On investigation, however, the former consignment was found to be at a too advanced stage, as the tetrad division had throughout been completed. The second batch of material, again, was too young, no stage beyond synapsis being discernible. In order not to delay the investigation further by correspondence under the present abnormal conditions prevailing in the postal service, I therefore fixed material myself, obtaining it from a garden at Marselisborg, near Aarhus (Jutland) in the month of July. The material was fixed in Carnoy's liquid, and stained with Delafield's haematoxylin. Besides the above mentioned species, I also fixed, in September, by the same means, some young flowers of *Lathyrus latifolius*, cultivated in the experimental nursery of the Carlsberg Laboratory, Copenhagen.

A cytological investigation, carried out essentially with a view to ascertaining the chromosome number, gave the following result.

Lathyrus odoratus L.

The chromosomes are large and somewhat elongated in the heterotypic metaphase. They are present to the number of 7 ($=x$) (Figs. 1 and 2). There is no difference, on the whole, in the size of the different chromosomes. Splitting and transition to the anaphase proceed fairly regularly. One of the chromosome pairs can, however, at times be separated slightly earlier than the remainder (Fig. 3). The heterotypic telophase may often give an impression that the chromosome number is greater than seven, owing to the fact that the chromosomes at this stage are bent to an angle, and also exhibit splitting in the plane through which the subsequent homoeotypic division takes place. The chromosomes being bent to an angle (the angle pointing toward the pole of the nuclear spindle) will in a certain position each appear as two separate chromosomes (schematically shown in Fig. 4) and as the chromosome is further split in the plane of the angle, we find, extremely often, apparent groups of four chromosomes, to the number of seven, i.e. 28 altogether (Fig. 5). Fig. 6 shows the chromosomes in the metaphase of the homoeotypic division; here also we can with great certainty count seven chromosomes in each of the two nuclear plates of the spore mother cell. In the anaphase of the homoeotypic division also, we may find what would seem like more than seven chromosomes, the chromosomes here being likewise bent to an angle, though not split.

An investigation of the chromosomes in somatic cells showed entire agreement with the figure found in the haplophase, i.e. 14 (Fig. 7). As is usually the case, the chromosomes here were of slenderer form than during reduction division.

Lathyrus latifolius L.

This perennial species appeared in every respect as *L. odoratus*, and the cytological picture for the two species is so uniform that preparations of the one might be taken for those of the other. Not only is the chromosome number likewise seven, as the heterotypic metaphase in Figs. 8-9 shows, but the chromosomes themselves are also entirely alike in size and shape. Here, as in *L. odoratus*, twice the number could be counted with perfect certainty in somatic cells (Fig. 10).

These two species, then—the only species of *Lathyrus* hitherto investigated—present an instance of the ordered regularity with which in larger or smaller systematic groups in the animal and vegetable

kingdoms, the chromosome members of closely related forms are found to be themselves related; i.e. either entirely alike, or multiples of a *cardinal number* characteristic of the group—as I have explained in my work above quoted.

The chromosome number seven is a comparatively low value to find among phanerogams, and should, *a priori*, count in favour of the employment of *Lathyrus* in genetic experiments. The theory as to agreement between reduction division and Mendelian segregation permits—commencing *either* with self-fertilisation of one heterozygotic individual *or* by crossing of two pure lines—that there be found at the outside seven independently segregating pairs of factors (or pairs of factor groups). A segregation experiment commenced in one or other of these two ways should not lead to the production of more than 128 different biotypes, all homozygotic throughout.

As to how far it may prove possible to find more than seven independently segregating pairs of factors or pairs of factor groups in a single biotype of the species, is a question which future investigations must decide. Personally I feel confident that they will *not* be found.

I have observed nothing in my material which might lead to the interpretation that chiasmatypy, in the sense of Janssens, is met with in *Lathyrus*. In my opinion, even in organisms in which breeding experiments have resulted in the view that parts of the chromosomes are exchanged during the reduction division, this process is not usually going on at so late a stage as shown by Janssens. More probably it occurs in synapsis where it cannot readily be demonstrated by microscopical methods.

CARLSBERG LABORATORIUM, COPENHAGEN,

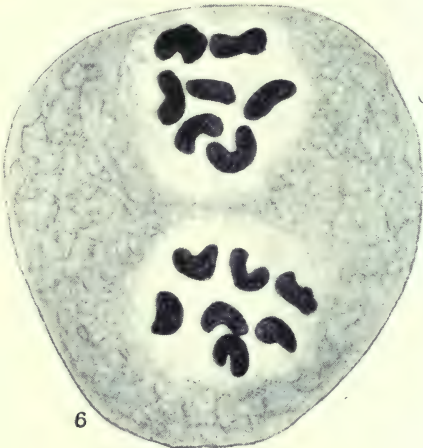
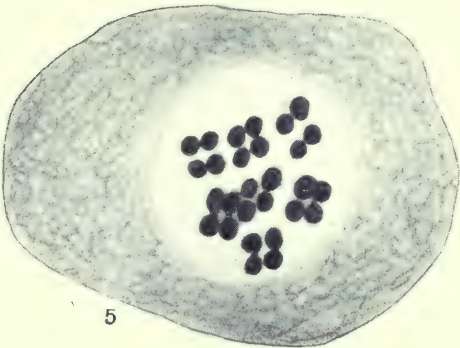
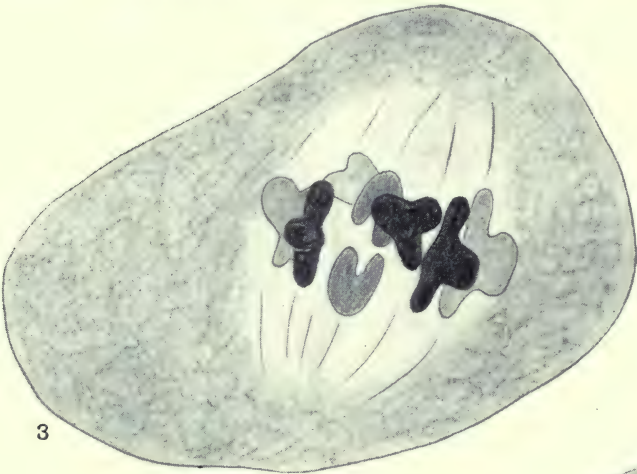
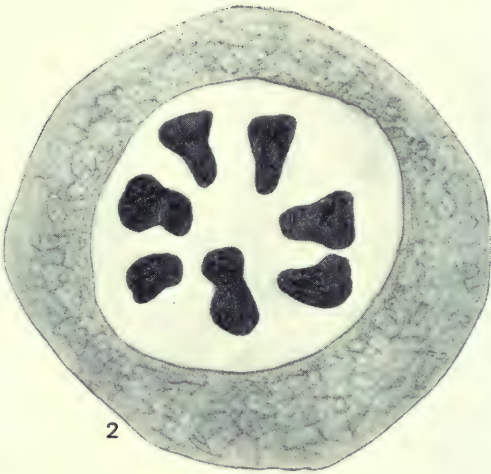
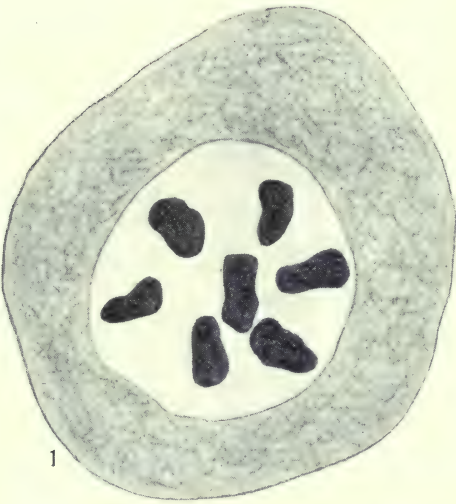
15 October 1918.

EXPLANATION OF PLATE V.

All figures are drawn with aid of Abbe's camera lucida, using Zeiss' homog. immers. 2 mm. and comp. oc. 18.

Figs. 1—7, *Lathyrus odoratus*. Figs. 8—10, *L. latifolius*.

- Figs. 1—2. Pollen mother cell. Heterotypical metaphase (polar view).
- Fig. 3. Pollen mother cell. Heterotypical meta-anaphase (side view).
- Fig. 4. Two chromosomes from heterotypical anaphase (schematically).
- Fig. 5. Pollen mother cell. Heterotypical anaphase (polar view). Apparently 7 groups of 4 chromosomes present.
- Fig. 6. Pollen mother cell. Homotypical metaphase.
- Fig. 7. Somatic cell with 14 chromosomes.
- Fig. 8. Pollen mother cell. Heterotypical metaphase (polar view).
- Fig. 9. Pollen mother cell. Heterotypical metaphase (side view).
- Fig. 10. Somatic cell with 14 chromosomes.





7



8



10



9

SEX SEGREGATION IN THE BRYOPHYTA.

By E. J. COLLINS, B.A. Camb., B.Sc. Lond.

Botanist to the John Innes Horticultural Institution.

(With Plate VI and Five Text-figures.)

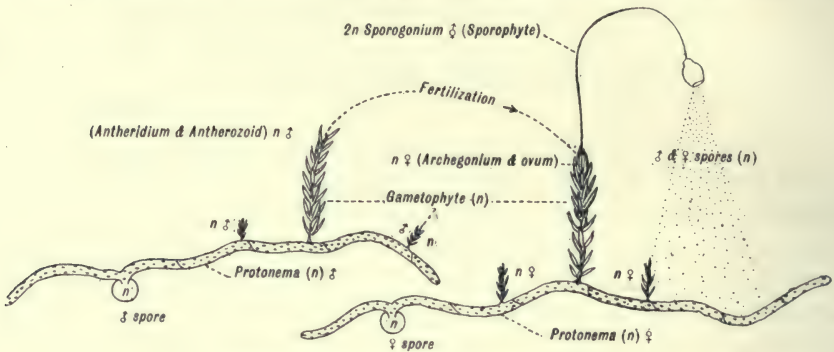
WHILST the majority of plants show an alternation of generations in the complete life cycle, it is in the Bryophyta alone that the gametophytic (n) phase shows an independent growth combined with a high degree of morphological differentiation. The study of sex in this group does not involve, as it does in the highest forms of plant life, the consideration of sporophytic tissues differentiated as "sex organs" in response to the parasitic nature of the gametophyte generation. It may well be that very considerable light would be thrown on the nature of sex and much information gained as to its mode of inheritance by further studies among the lower plant groups.

In recent years no more interesting and suggestive papers concerning sex in Mosses have been published than those of El. and Em. Marchal¹, and a very brief outline of these papers will serve to show the scope of their researches.

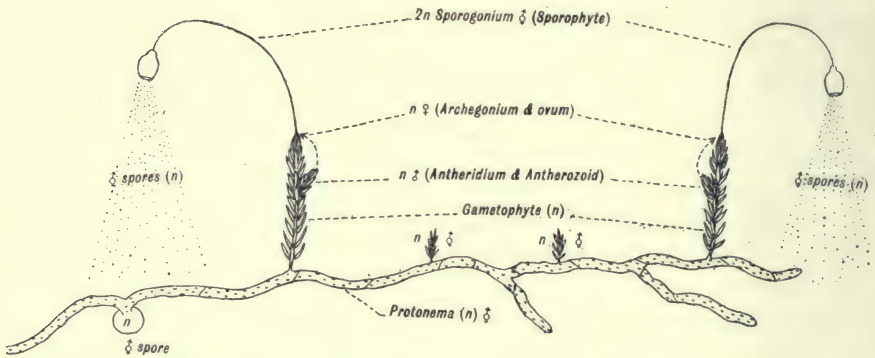
By compounding a suitable culture fluid, these investigators have grown what might be regarded as pure line cultures of the various mosses experimented with. Starting with the spores from capsules of dioicous forms, these were shown to be uni-sexual, inasmuch as the protonemata developed from them produced leafy axes which were all of one sex, either male or female. The male and female axes so produced were approximately equal in number, and the protonemata obtained vegetatively from them gave leafy axes of the same sex as the parent axes. Protonemata were then induced to develop aposporously from sporophytic ($2n$) tissue, and these produced a proportion of hermaphrodite axes. The proportion of hermaphrodite axes however was very low; e.g. in *Bryum caespiticium*, of 1738 axes 1579 were male, 154

¹ *Mém. de l'acad. roy. de Belgique*, 1906; *Bull. de l'acad. roy. de Belgique, Classe des Sciences*, 1907, 1909, 1911.

hermaphrodite and 5 female, a result which shows a striking preponderance of male axes. No sporogonia were borne upon any of the aposporous products as the sex organs were sterile. Abnormal organs of mixed sex also were found in *B. caespiticius* and *Mnium hornum*. The investigators concluded that there was cause to think that the uni-sexuality of dioicous mosses in the haploid phase was absolute, and due to the presence—to the exclusion of the other—of only one sex determinant, and that the



Diagrammatic Life Cycle of Dioicous Type.



Diagrammatic Life Cycle of Monoicous Type.

maturation process was the occasion of the segregation to the state of purity of sexual characters in the spores. In other words a clean cut sex segregation occurred at the reduction division of sporogenesis. Undoubtedly the low proportion of hermaphrodite axes resulting from aposporously developed protonemata is a serious hindrance to the acceptance of the theory, for on the hypothesis we should have expected that plants arising aposporously from sporogonial tissues would all be

hermaphrodite. The difficulty is recognised by El. and Em. Marchal, but in alleviation of this objection they suggest that among the male plants may have been many in which the development of ♀ organs may have been suppressed. The aposporous products of the dioicous forms *B. caespitium*, *B. argenteum*, *B. capillare*, *B. fallax* and *M. hornum* were without exception sterile. Aposporous products of monoicous forms were fertile, producing diploid gametes. These united in fertilisation, and by further aposporous development from the sporogonium tetraploid gametophytes were formed.

In support of the theory of sex segregation at sporogenesis the recorded sex behaviour of *Sphaerocarpus terrestris* is often quoted. As is well known, the spores of this Hepatic are shed in the original tetrad groups and according to C. Douin, two ♂ and two ♀ plants result from the development of the spores of each tetrad. More precisely, of 81 spore groups examined 64 showed two ♂ and two ♀ plants, 13 germinated imperfectly and 4 showed results not in agreement with expectation.

Recently Allen¹ has reported a chromosome difference correlated with sex differences in *Sphaerocarpus Donelli*, and his limited observations indicate that of the spores of each tetrad, two give ♂ and two give ♀ plants as in *S. terrestris*. At the reduction division two of the four spores each receive a large chromosome and these develop female plants, whilst the other two receive a small chromosome each and give rise to male plants.

It will be seen that the theory emphasises the dual nature of the sporophyte generation, in that it carries both sex determinants. The determinants however bring about no expression of sex in the sporogonium, but assuming that dioicous sex plants were foreshadowed by two types of spores, a differentiation of sporophytic tissues in conformity with the change might ensue. Thus changes would be initiated which would give the sporophyte the appearance of "sex monoeism," and inhibition of the differentiation of one or other of the "sex organs" would lead to the dioicous sporophyte. Should the relative importance of the two generations in the life cycle become reversed, the parasitism of the sexed plants would bring about a still greater differentiation of tissues of the sporophyte in accordance with the physiological need. Needless to say the changes would lead to the impression of "sex characters" upon the sporophyte.

Scheme I is suggested as an expression of the theory for dioicous forms, whilst Scheme II would apply to monoicous types. In the latter

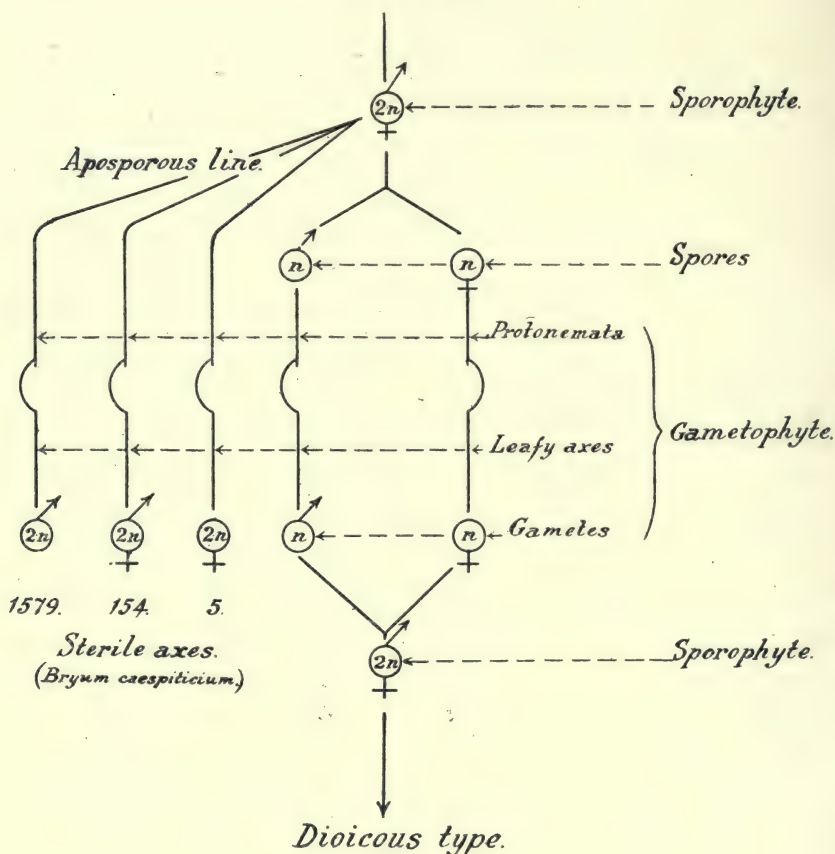
¹ *Science*, N.S. Vol. XLVI. 1917. p. 466.

scheme an alternative reading is given (*A* and *B*). If the purity of the gamete for sex characters be maintained, a sex segregation in the somatic tissues of the gametophyte is assumed to occur (*B*). This point will be considered a little later.

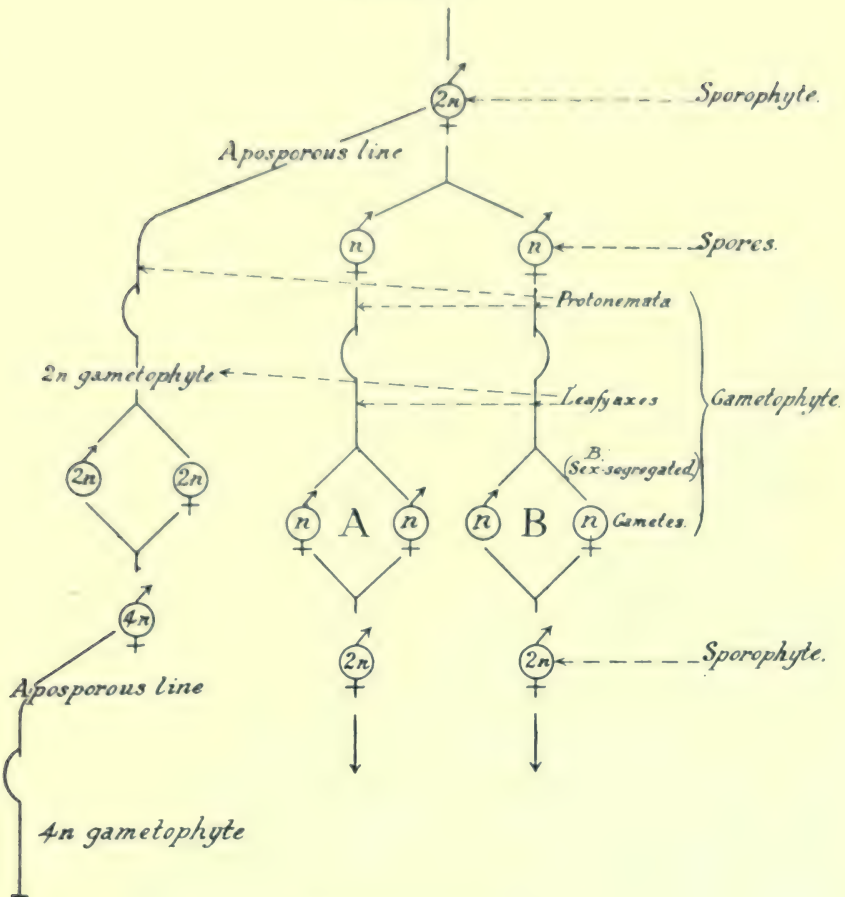
Quite naturally the question arises, how far can the facts of sex inheritance gained from the study of animals, be applied to these plant forms, if indeed they can be applied at all? At the outset it must be borne in mind that in general, meiosis accompanies gametogenesis in animals, and sporogenesis in plants. Exceptions occur in plants, as for example in the Fuci, where there is no alternation of generations and reduction occurs at gametogenesis.

Assuming that in a monoicous (ϕ) form of moss, the gametophyte produced σ gametes bearing the male factor, and ϕ gametes bearing

SCHEME I.



SCHEME II.



Monoicous type.

either the ♂ or ♀ factor, then two types of sporogonia would be produced. (a) those bearing exclusively male elements whose spores (♂) would result in ♂ gametophytes only and (b) those carrying both male and female factors whose spores (♀) would give rise to monoicous (♂♀) gametophytes. Such a possibility is not excluded¹, although it is generally assumed, without definite knowledge to the contrary, that the

¹ In *Bryum mamillatum* the monoicous condition is accompanied by distinct male plants. It is not possible to say whether such plants have been derived from spores produced by sporogonia exclusively male in character, or whether of the spores in any one capsule some bear ♂ and others ♀ factors.

spores of any moss capsule of a monoicous species produce monoicous gametophytes. The further assumption, that maleness is recessive, would mean the extinction of the monoicous habit.

Again in dioicous species it is generally agreed that the spores of any single capsule develop to give rise to either ♂ or ♀ axes, but the theory would lead us to assume, as with monoicous forms, the existence of certain capsules, all the spores of which produce male axes exclusively, and others whose spores produce monoicous axes in which maleness is latent.

Although the experiments upon which El. and Em. Marchal based the theory of sex segregation have not been repeated, the theory has been subjected to considerable criticism. Recently M. Wilson¹ found organs of mixed sex together with normal sex organs upon naturally growing plants of *Mnium hornum*. A cytological examination was made of this material and a normal antheridium which happened to be in spermatogenesis showed the haploid number of chromosomes. From this it was evident that meiosis had occurred, and it was not probable that the plant had been produced aposporously as the theory would demand. Also it was suggested that a low proportion of hermaphrodite axes, such as was produced in aposporous cultures, might very well be found in nature following a normal process of sporogenesis.

My own experience relates to the well-known form *Funaria hygrometrica* which has been described by many competent observers as monoicous, whilst others, equally competent, have described it as a dioicous species. Under these circumstances Boodle² some years ago undertook an inquiry into the question of the distribution of the sex organs, and after examining material from many localities came to the conclusion that the plant was monoicous, and dioicism, if it occurred, was comparatively rare. It was found that the shoot which bore archegonia terminally, arose as a lateral branch of the axis bearing the terminal male "flower," and no instance was observed in which a female axis produced a male branch. Boodle concluded that the dioicous habit was attributed to the plant because young plants bearing the discoid male "flowers" only were found, and that if female branches were gathered they were generally torn from the male axis to which they were attached, the presence of a basal tuft of rhizoids on the female branches giving them the appearance of distinct plants.

During the course of some experiments conducted for the purpose of determining the best cultural conditions for the production of proto-

¹ *Annals of Botany*, 1915.

² *Annals of Botany*, 1906.

nemata from various moss organs and spores, three cultures of *Funaria hygrometrica* were made in Marchals' nutrient liquid. These cultures were obtained (a) from antheridia taken from a single male "flower," (b) from perigonial leaves, and (c) from the spores shed from one ripened capsule. Each culture when sufficiently grown was poured out upon soil contained in a small earthenware pot, over which boiling water had been poured some little time previously. The pots were then covered and stood in a cold frame. When the protonemal felt was observed growing strongly, the coverings were removed and no further attention was given to the pots. Later however it was observed that whilst the sward of plants obtained from the culture from spores had produced a dense crop of sporogonia, a close sward of plants with large discoid male "flowers" had appeared in the two cultures resulting from antheridia and perigonial leaves. A second crop of discoid male "flowers" was produced by these same cultures but no sporogonia were produced at any time¹. Photographs of two of these cultures, A from spores and B from antheridia, are reproduced on Plate VI.

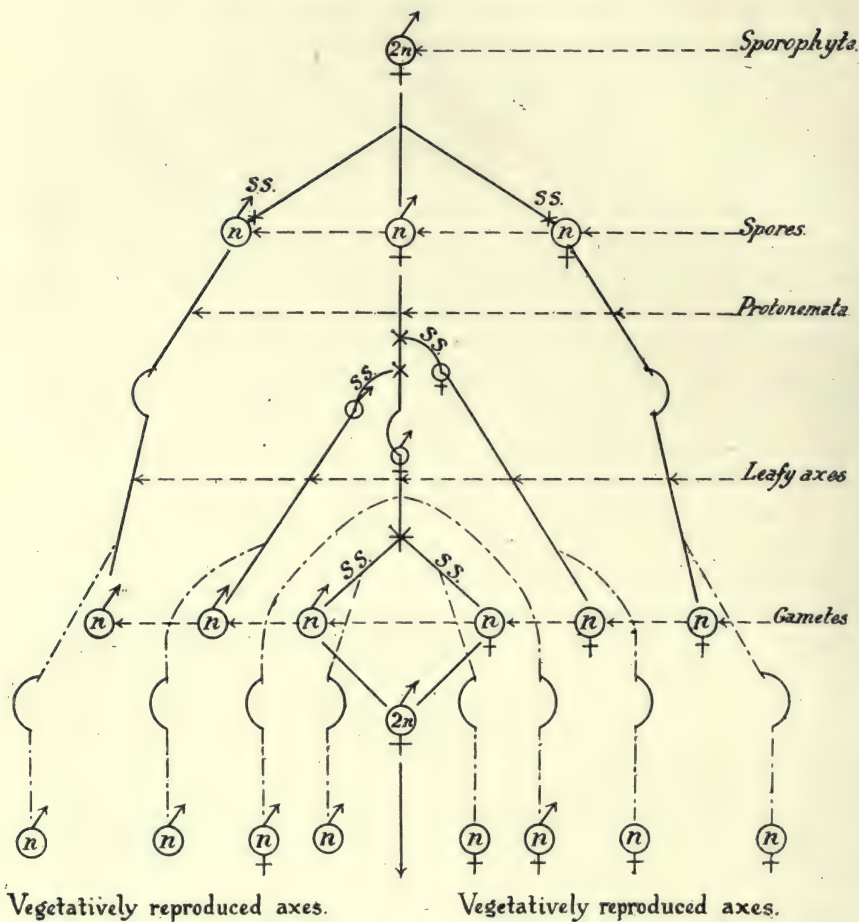
It appears possible that vegetative development from structures borne on male and female axes respectively may, if a sex segregation has actually occurred somatically, lead to the production of distinct male and female plants. If such is the case *the purity of the gamete in monoicous forms is secured by a somatic segregation in haploid tissue*. It is interesting to recall in this connection that El. and Em. Marchal have stated for *Funaria*, that ♂ axes and ♀ axes may arise from the same protonema and that this has led to a confusion concerning monoicous and dioicous forms. Thus the point at which segregation occurs is not necessarily fixed, but may be shifted backward in the life cycle until it occurs at sporogenesis. Thus the dioicous habit of the gametophyte may, as we can imagine, have been established. In this way the dioicous condition might co-exist with the monoicous (autoicous), a state of affairs known to occur in some mosses, e.g. *Dicranella crispa*, or the autoicous condition might be accompanied by distinct male plants e.g. *Bryum mamillatum*.

Other forms might be mentioned which show varying sex conditions, whilst vegetative propagation from sex-segregated axes would also lead to the production of the various sex-forms of one species.

The generalized scheme figured on p. 146 deals with sex segregation and vegetative reproduction in both monoicous and dioicous types.

¹ Because of this interesting result, perichaetial leaves associated with the archegonia in *Funaria* are now being made the subject of a similar experiment.

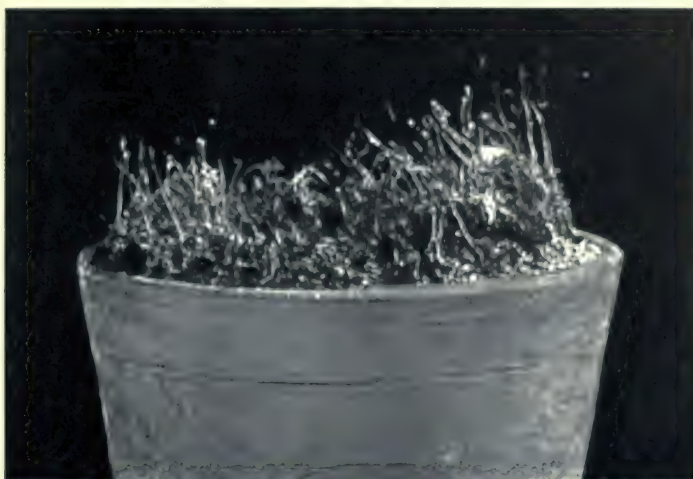
GENERALIZED SCHEME.



* SS. Point at which it is assumed that a segregation of sex occurs.

PLATE VI.

For explanation see p. 145.



A. Plants grown from spores. Monoicous plants bearing sporogonia.



B. Plants grown from antheridia. Males only.

RACIAL STUDIES IN FISHES

II. EXPERIMENTAL INVESTIGATIONS WITH *LEBISTES RETICULATUS* (PETERS) REGAN

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(With One Graph.)

I. *Introduction.*

The purpose of the experiments about to be discussed was to contribute information on the rather obscure question whether, or to what extent, quantitative racial characters are hereditary.

The tropical-American Cyprinodont *Lebistes reticulatus* (Peters) Regan¹ was employed in the experiments. I have previously used this little aquarium-fish in experimental investigations, namely for the purpose of demonstrating the importance of environment on the numbers of organs (dorsal rays).

Lebistes reticulatus is, like so many of its relatives, *viviparous*, and under favourable conditions the female brings into the world, at intervals of about 4 weeks, a considerable number of young. The young possess at birth the full number of vertebrae, dorsal rays, etc., which is therefore recognisable immediately after birth.

The experiments fall into two groups, of which the first helps to elucidate the importance of *external factors* (temperature) upon the number of dorsal rays. The second is concerned with the question whether hereditary differences, i.e. differences dependent upon *internal factors*, may be proved to exist in different individuals. Before I proceed to discuss the experiments, I may draw attention to the fact that the

¹ C. Tate Regan, "A revision of the Cyprinodont Fishes of the Subfamily Poeciliinae," *Proc. Zool. Soc. London* 1913, Vol. II. pp. 977—1018, 1913.

number of dorsal fin rays in *Lebistes reticulatus* varies from 5 to 8. By far the most usual number is 7.

A more detailed account appears in Vol. XIV, Nos. 1 and 5, of the *Comptes-Rendus des Travaux du Laboratoire de Carlsberg*, Copenhagen.

II. Importance of External Factors.

The principle was to vary the temperature for the same pair of parents from one period of pregnancy to the other, and then determine the number of dorsal rays in the various broods of offspring. In the beginning I had no means of maintaining a constant temperature in the aquaria, and was therefore compelled to limit myself to stating that the animals in the experiments were kept at a "low," "medium" and "high" temperature, in which "medium" temperature was ca. 6° above "low" and ca. 3° below "high." "Low" temperature was generally equivalent to ca. 19° Centigrade, varying between ca. 17° and ca. 23°.

In the experiments 5 different pairs of *Lebistes reticulatus* were used. The results of these investigations can be seen from Tables I—V, each of which shows the number of rays in several broods from the

TABLES I—V. Number of dorsal rays in offspring of the same pairs of parents at different temperatures.

TABLE I. ♂ 7 × ♀ 7.

No. of rays	High temperature Born 12 March	Medium temperature Born 13 April	Low temperature Born 1 June	Low temperature Born 25 July	High temperature Born 25 Sept.
8	9	—	—	1	4
7	6	20	25	33	16
6	—	—	13	4	—
n	15	20	38	38	20
a	7.600	7.000	6.658	6.921	7.200
σ	± 0.532	—	± 0.493	± 0.390	± 0.414
P. E. A.	± 0.093	—	± 0.054	± 0.043	± 0.062
Fl.	± 0.465	—	± 0.270	± 0.215	± 0.310

TABLE II. ♂ 8 × ♀ 8.

No. of rays	Medium temperature Born 12 April	Low temperature Born 29 May
8	15	—
7	29	27
6	—	5
n	44	32
a	7.341	6.844
σ	± 0.483	± 0.399
P. E. A.	± 0.049	± 0.048
Fl.	± 0.245	± 0.240

TABLE III. ♂ 8 × ♀ 7.

No. of rays	Medium temperature	Low temperature
	Born 23 May	Born 14 July
8	8	—
7	49	31
6	—	6
n	57	37
a	7.140	6.638
σ	± 0.351	± 0.399
P. E. A.	± 0.031	± 0.044
Fl.	± 0.155	± 0.220

TABLE IV. ♂ 6 × ♀ 6.

No. of rays	Medium temperature	Medium temperature	Low temperature
	Born 1 April	Born 27 May	Born 13 July
8	1	1	—
7	11	31	15
6	3	6	21
n	15	38	36
a	6.867	6.868	6.417
σ	± 0.565	± 0.439	± 0.505
P. E. A.	± 0.098	± 0.048	± 0.057
Fl.	± 0.490	± 0.240	± 0.285

TABLE V. ♂ 6 × ♀ 6.

No. of rays	Medium temperature	Low temperature	Low temperature
	Born 7 May	Born 19 June	Born 14–15 August
8	—	—	—
7	11	17	35
6	—	11	18
5	—	2	—
n	11	30	53
a	7.000	6.500	6.660
σ	—	± 0.636	± 0.487
P. E. A.	—	± 0.078	± 0.045
Fl.	—	± 0.390	± 0.255

same pair of parents. The date of birth of the young is noted in each case, as also whether developed at low, medium, or high temperature.

It is distinctly evident from the tables that the different broods do exhibit a difference in the number of dorsal fin rays, and it is further seen that the average number of rays was greater where the young had been developed at a high temperature than where their development took place at a low temperature.

In all my later experiments this result has been confirmed. I will content myself with discussing a single one of the later experiments, which, technically speaking, had the advantage over the preceding ones in that the temperature in the aquaria could be kept constant, there

being a fluctuation of only one-tenth of a degree (± 0.1). The two parents had respectively 7 and 5 rays in the dorsal fin. The experiment took place partly at 25° , partly at 18° . The three first broods were produced at 25° , following which the parents were maintained at 18° , from the day the third brood was born until the birth of the fourth brood. After this the temperature was raised again to 25° , at which degree the development of the last broods of young took place. The result of the experiment is given in Table VI.

TABLE VI.

Number of dorsal rays in offspring of the same pair of parents at different temperatures.

No. of rays in offspring	No. of Specimens					
	25° Brood 1 Born 15/5 1918	25° Brood 2 Born 10/6 1918	25° Brood 3 Born 6/7 1918	18° Brood 4 Born 21/9 1918	25° Brood 5, 6, 7 Born 25/10, 21/11, 24/12 1918	25° Brood 1, 2, 3, 5, 6, 7
7	8	13	29	6	51	101
6	1	2	3	13	4	10
5	—	—	—	1	—	—
n	9	15	32	20	55	111
a	6.889	6.867	6.906	6.250	6.927	6.910
σ	± 0.333	± 0.352	± 0.296	± 0.550	± 0.262	± 0.289
P.E.A.	± 0.075	± 0.061	± 0.035	± 0.083	± 0.024	± 0.018
Fl.	± 0.375	± 0.306	± 0.177	± 0.415	± 0.129	± 0.092

Thus we see, that whilst the broods developed at 25° had an average of 6.91 rays, the average number of rays fell to 6.25 at 18° . The difference between the averages was thus 0.660 and the probable error of this difference ± 0.085 .

As all experiments in this connection have given a similar result, it may be taken as proved that the number of rays in the dorsal fin of the offspring is *affected to a considerable degree by the temperature* to which the mother is subjected whilst in a state of pregnancy. Remarkable besides is the great difference in the duration of pregnancy at the different temperatures; which at 25° lasted ca. 1 month, at 18° more than 3 months.

III. *Importance of Internal Factors.*

The object of the experiments about to be discussed was to investigate whether hereditary differences in the number of dorsal fin rays could be proved to exist. The principle of the experiments was to maintain *different* pairs of parents in the *same* environment, and see whether the offspring were different as regards the number of rays.

The specimens employed in the experiments were selected from two races with which since 1915 selection experiments had been undertaken, partly towards a high, partly towards a low number of dorsal rays. The parents had, respectively, both 8 and both 6 rays in the dorsal fin. In each case the specimens were kept at a constant temperature, viz. 25°. In addition, in order to secure uniform environment, the specimens whose offspring should be compared were placed in the same aquarium, separated only by a trelliswork of thin glass tubes. In other words they lived in quite the same body of water, maintained at a constant temperature. The aquarium contained no plants, but a continuous stream of atmospheric air bubbled through the water.

The experiment falls into two series, A and B. In series A there were employed partly ♂ 269 and ♀ 270 (each with 6 rays), partly ♂ 267 and ♀ 268 (each with 8 rays). For series B there were employed partly ♂ 274 and ♀ 273 (each with 6 rays), partly ♂ 276 and ♀ 275 (each with 8 rays). All the experimental fish in series A were kept in the same aquarium which stood at the side of the one in which all the fishes belonging to series B were placed.

From the appended Table VII and from the graph on p. 152 one remarks that there was in both series a very great difference in the

TABLE VII.

Number of dorsal rays in offspring of four different pairs of parents at a constant temperature of 25° C.

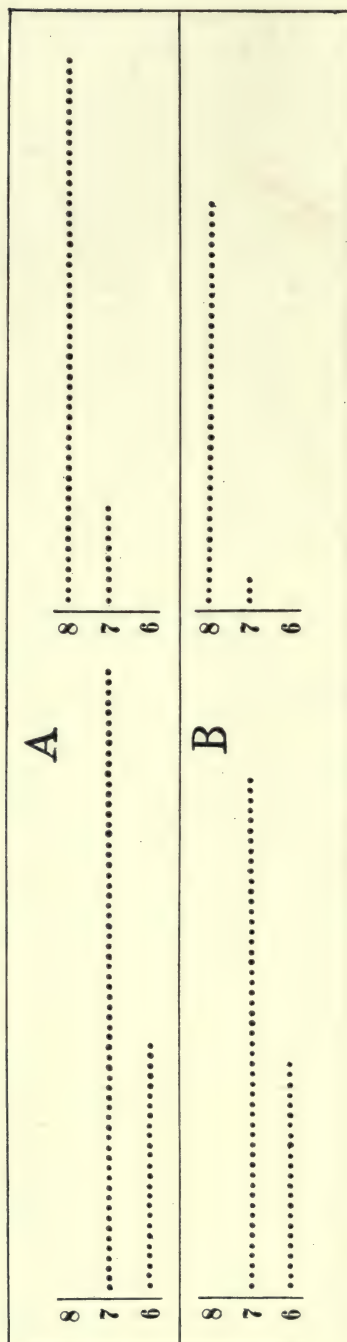
No. of rays in offspring	Series A		Series B	
	♂ 269 × ♀ 270 both 6 rays	♂ 267 × ♀ 268 both 8 rays	♂ 274 × ♀ 273 both 6 rays	♂ 276 × ♀ 275 both 8 rays
	Brood 1, 2, 3, 4	Brood 1, 2, 3, 4	Brood 1, 2, 3, 4	Brood 1, 2, 3
	Born 23/9, 17/10, 9/11, 4/12 1918	Born 27/9, 23/10, 19/11, 17/12 1918	Born 29/9, 24/10, 21/11, 18/12 1918	Born 29/9, 26/10, 26/11 1918
8	—	54	—	40
7	62	10	51	3
6	25	—	23	—
n	87	64	74	43
a	6·713	7·844	6·689	7·930
σ	± 0·455	± 0·366	± 0·466	± 0·258
P. E. A.	± 0·033	± 0·031	± 0·037	± 0·027
Fl.	± 0·165	± 0·154	± 0·183	± 0·133

average number of rays in the offspring of fishes with 6 and with 8 rays, namely in the first series **1·131** (Probable error of difference = 0·045) and in the second **1·241** (P.E. Diff. = 0·045).

This difference cannot be due to difference in environment because the fishes swam in the same aquarium, indeed in the very same water

♂ 6 × ♀ 6

♂ 8 × ♀ 8



EXPLANATION OF THE GRAPH.

Number of rays in the dorsal fin in offspring of 4 pairs of parents all kept at 25°. Graphical representation of the experiment given in Table VII.

The figures give the number of rays; each dot represents one individual member of the offspring. The two upper graphs refer to Series A; the two lower ones to Series B of the experiment. The two graphs on the left represent offspring of parents having 6 rays in the dorsal fin, the two on the right represent parents with 8 rays.

It is to be seen that in each series the offspring is different in spite of the environment being identical.

at a constant temperature and with regular ventilation. The conditions with regard to uniformity were in my opinion the most favourable possible and I cannot but conclude from the present experiment, that the *difference proved to exist in the offspring of parents with respectively 6 and 8 rays is of hereditary (genotypical) nature.*

IV. *Concluding Remarks.*

The investigations here treated fall into two groups: (1) Experiments in which the *same mother* was exposed to *different environments* in different periods of pregnancy and (2) Experiments in which *different mothers* were exposed to the *same environment*, have thus succeeded in elucidating these rather complicated questions.

It has been shown that the number of organs may be very susceptible to environment, but that this fact cannot—under suitable experimental conditions—disguise the fact, which we specially wanted to demonstrate, viz. that there are or may be differences of hereditary nature between the various individuals.

This proof is of considerable interest for our view upon the nature of "races" in fishes, and supports in a high degree the opinion expressed by me at a previous occasion¹: "My view then, with regard to the nature of 'races' in fishes, as characterised by our population analyses, is briefly this: A fish 'race' is largely a statistical conception. It implies a mixing of different genotypes, and the average values characterising the 'race' are primarily dependent upon the quantitative proportion between these; only secondarily on the environment."

¹ Johs. Schmidt, "Racial Studies in Fishes. I. Statistical Investigations with *Zoarces viviparus*, L.," *Journal of Genetics*, Vol. VII. p. 117, 1918.

CROSSING THE NORTH AFRICAN AND SOUTH AFRICAN OSTRICH.

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(With Plate VII, and Two Text-figures.)

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INTRODUCTION.

THE continent of Africa, with the adjoining parts of Arabia, Palestine and Asia Minor, is the natural home of the ostrich genus *Struthio*. Beyond the confines of Africa however the wild bird is now extremely rare, if it exists at all; while in Africa it is slowly passing away as the continent becomes occupied by the white settler. The domesticated bird on the other hand has greatly increased in number during the fifty years of ostrich farming, amounting to near one million in 1913, though since considerably reduced owing to the less demand for plumage as a result of the prolonged war.

Zoologists recognise four species of the two-toed ostrich: the North African ostrich, *Struthio camelus* Linn., the South African ostrich *S. australis* Gurney, the East African ostrich, *S. massaicus* Naumann and the Somali ostrich, *S. molybdophanes* Reichenow. The two last mentioned are not however well-established species, appearing to represent intermediate types of the other two. On the other hand the northern and the southern birds have well-defined characteristics separating them, connected with size, colour, nature of the egg and other minor features. Observing them side by side no one would hesitate in assigning them specific distinction.

Recently a unique opportunity has presented itself for studying numbers of the northern and southern ostrich under similar conditions, and also the behaviour of their characters when the two are crossed. In 1912 the Government of the Union of South Africa imported 132 specimens of the North African ostrich from Nigeria¹, with the object of possibly improving the domesticated strains built up from the original South African wild bird. The imported birds were stationed at the Grootfontein School of Agriculture, and the breeding experiments to be conducted with them were placed in charge of the writer.

The main object of the investigations is the practical one of determining to what degree the plumage of the southern bird can be improved by crossing with the northern, but in the course of the work many other questions have arisen which have an interest to students of genetics generally. The experiments have been in progress for over four years, and during that period about a hundred cross-bred chicks (F_1) have been hatched as well as a score or so of pure North African chicks; at the present time some of the first crosses have reached the age at which they are beginning to breed, but only two chicks belonging

¹ Report on the North African Ostriches imported into South Africa in 1912. Union of South Africa, Department of Agriculture, Pretoria, No. 2, 1916.

to the second hybrid generation (F_2) have yet been reared. The earlier matings were carried out with whichever of the northern birds happened to attain sexual maturity, irrespective of their plumage qualities or other characters; but with the abundant material now available crosses are made with a definite purpose in view. The long period between the maturity of one generation and the next, usually between three and four years, necessarily renders progress slow¹.

The distinguishing characters of the northern and the southern ostrich are as follows:

A. The North African Ostrich, *Struthio camelus* Linn. The species is larger and stronger than the South African bird, the head reaching about 8½ feet from the ground; the length of the body from the tip of the beak to the end of the tail is about 8 feet, and the total weight about 275 lbs. The neck is also longer, about 3½ feet in length, and the body feathers extend upwards for about 1¾ feet. The legs are longer, thicker and more robust, the knee joint being at least 4 feet from the ground, and the feet are larger; a claw is sometimes present on the small toe and the scales over the large toe may show one or, rarely, two breaks.

The number of wing plumes, or remiges, is about 36 to each wing, but varies from 33 to 39.

The colour of the skin in immature birds of both sexes, as well as of mature hens, is a creamy yellow, while the mature cock is bright red or scarlet on the legs, head and neck, and red or pink over the body generally.

The crown of the head has a bald patch, either single or partly divided (Text-fig. 1, p. 158).

The egg is smooth, as if polished, practically free from deep pittings or pores, and larger and more rounded than that of the southern bird. The average long diameter is 6.15 inches and short diameter 5.35 inches and weight 3 lbs. 11 oz. (Pl. VII, fig. 3).

Found in Northern and Western Africa, and in times past ranged eastwards to Abyssinia, Arabia and South Palestine.

B. The South African Ostrich, *Struthio australis* Gurney. Smaller than the North African, the less size being due to the slender and shorter legs and neck rather than to any difference in the size of the

¹ The wild ostrich breeds when about four years old but the domesticated bird, largely as a result of high nutrition as a chick and young bird, along with a certain amount of unconscious selection, now usually breeds when between two and three years old, though chicks are sometimes hatched from birds under two years.

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body. The head extends about $7\frac{1}{2}$ feet from the ground, the length from the tip of the beak to the end of the tail is $7\frac{1}{2}$ feet, and the total weight about 240 lbs. The neck is about 3 feet long, and the body feathers pass upwards for about 1 foot; the knee joint is about $3\frac{1}{2}$ feet from the ground; a claw is rarely present on the small toe, and the scales on the tarsus and third toe are usually continuous.



Text-fig. 1. Head of North African ostrich showing baldness. The oval area towards the hinder border represents the pineal spot.

The wing plumes average about 36 to each wing, but vary from 33 to 42.

The skin of the neck, body and legs is pale yellow in chicks, dark grey in the mature hen, and dark blue in the cock. In the sexually ripe cock only the beak, front part of the head, naked skin round the eyes and tarsal scales are a bright scarlet, and the rest of the tarsus and toes pink, but the redness does not extend above the ankle.

The crown of the head is without any bald patch, and often bears a tuft of longer hair-like feathers in the middle.

The egg is deeply pitted all over, smaller and more oval than in the northern bird. The average long diameter is 6 inches and the short diameter 5 inches; the weight about $3\frac{1}{2}$ lbs. (Pl. VII, fig. 3).

Inhabits practically the whole of the sub-continent of South Africa.

Observed alongside one another, as can now be done at Grootfontein, no one could fail to distinguish the northern from the southern ostrich. The greater height of the former, the stronger limbs and the light yellow of the skin are obvious features, while the sexually mature cock is still more distinctive on account of the brilliant scarlet of the head, neck and legs, and the red colour of the body generally. The naked patch on the head and the smooth, larger eggs are just as constant distinguishing features. Many small differences of feather structure occur, and are of much importance to the practical ostrich farmer. Hitherto all the distinguishing characters of the imported northern ostrich have been retained under the new conditions of South Africa and re-appear in the progeny, showing that they are hereditary and independent of climatic and other environmental influences.

Whether the northern and the southern ostrich are to be regarded as distinct species depends largely upon one's conception of the term species and will be discussed later. It may be noted however that the two are found to interbreed freely and reciprocally, and the crosses or hybrids have also been proved to be fertile, both *inter se* and with either of the parent forms; at the same time the two races have many distinctive characters which are germinal in their origin.

NATURE OF THE MATERIAL.

From a genetical point of view the material on which the investigations are being conducted is heterogeneous. The imported Nigerian birds are such as were procured by the Arabs from wild nests, and then reared in kraals for the sale of their plumage. Methodical ostrich farming, as conducted in South Africa, is scarcely known in North Africa, and chicks are not bred in captivity; hence the birds are uninfluenced by any artificial selection. They exhibit much variation in the details of feather structure, and many distinct commercial types of plumes are represented, though all the birds come from one area. On importation the greater number were chicks about six months old. The majority of the older birds failed to become adapted to their new environmental conditions, and many died from lack of immunity to the various parasitic diseases affecting the southern bird. A selection has been made of those producing the most desirable plumage and the others discarded.

The South African birds employed are such as have been produced by gradual selection during the fifty years or so since ostrich farming was first established at the Cape. The foundation stocks naturally consisted of wild birds, and the best of these and their progeny have been employed in building up the superior strains of to-day. The ultimate object in ostrich breeding is simple and well defined. The farmer selects only for feather production; no other character of the bird is taken into account, as any weakness in constitution or breeding power is scarcely observable. Also, practically only one feather ideal exists, namely, the largest feather combining all the many desirable features at their maximum. The technical "points" relate to details concerning length, width, strength, shapeliness, density and lustre, in all of which ostrich plumes vary greatly. None of the original South African strains possessed the highest expression of these in combination, and the object throughout has been to gather into one all the best features available. As yet no breeder has succeeded in doing this, though many are nearing the desired end. The ostrich farmer clearly appreciates the distinctness of the various characters of the plume, though in his selection for mating he proceeds mainly on the assumption of a blending inheritance, and in practice the method is succeeding, even though progress is slow and much variety is encountered in the progeny. When the ideal type of plume has been built up it is understood that it must be "fixed" by a measure of in-breeding, and at present widely divergent crosses are rarely made.

It is generally conceded that, notwithstanding all the selection which has taken place, no advance has been made on the best of the feather points originally scattered among the foundation stocks, except such as can be ascribed to improved nutrition and other conditions dependent upon domestication¹. All that the farmer has done is to combine in the one plume the best of the features originally distributed

¹ As long projecting outgrowths of the epidermis, with a core or medulla of highly vascular nourishing dermis, ostrich plumes during their six months' growth are remarkably sensitive to the nutritive and other physiological conditions of the bird; even the variations of blood-pressure between day and night often leave their impress upon the growing feather in the form of "bars," while climatic conditions may make or mar the success of the feather crop. The greatest difference in value obtains between a well-grown and an imperfectly grown crop of feathers, and this accounts for the great care bestowed upon the management of the birds, and the highly stimulating food supplied. Probably the high grade ostrich is the best cared-for and most pampered of all our domestic animals. No better subject for studying the influence of a varying nutrition and blood-pressure upon a growing structure could be chosen than its long plumes, growing at the rate of a quarter of an inch a day.

among the many wild strains; but it has been found impossible to change any of the characteristics beyond what nature provided. Taking the features separately the ostrich plume affords a noteworthy instance of the impossibility of improvement, beyond the maxima originally present, by means of continued selective breeding. All that the process has achieved is to segregate the characters most desired; moreover, in connection with the points of the feather no hint of any sport or mutation ever occurs.

When comparing closely the many commercial varieties of ostrich plumes, each representing a separate type to the specialist and having a distinct value, the question arises as to how far the many differences in size, density, shape and lustre should be considered as fluctuating variations, or how far they are elementary characters. Farming experience has fully proved that selective breeding within a type will not change any of the minutiae of the type. If a farmer requires any particular feature added to his strain he must procure it from birds whose plumage displays it; no degree of breeding will otherwise produce it. It is thus shown that the variations distinguishing the types are germinal, not environmental, and should therefore be regarded as representing definite elementary characters. Yet how many of these elementary characters must be represented in even a single ostrich plume! No doubt the same multiplicity of small characters appeals to the specialist in an intensive study of any domesticated stock. Experimental investigations are usually undertaken on one or on only a few of the pronounced characters, but when all the many details have to be considered which are of the greatest importance to the practical breeder, there appears no limit to which analysis can be carried¹.

The present paper is confined to the behaviour in crosses of certain of the more prominent characters which distinguish the northern and southern ostrich, such as the dimensions, colour, the bald head patch and details connected with the egg, and others which the two have in common, such as the number of plumes on the wing, the scales on the middle toe and the claw on the fourth toe. An attempt is made to arrive at the germinal constitution of the ostrich so far as concerns these characters, as well as their adaptive value and manner of establishment in the race.

¹ Two preliminary attempts have already been made to analyse the various plume characters of the parents and progeny in cross-matings, *Breeding Experiments with North African and South African Ostriches*, I. "The Plumes of Parents and Chicks"; II. "The Plumes of the Second and Third Clippings," Local Series, Nos. 13 and 17, Department of Agriculture, Union of South Africa, 1917.

DIMENSIONS.

On account of its restless nervous nature and the difficulty of fixing upon constant determinable points the live ostrich is not a creature which lends itself to accurate bodily measurements. In any troop it will be found that the members differ much among themselves, and the same individual varies at different ages and according to its nutritive condition. Hence the northern and southern birds and the crosses from them can be compared only in general terms, as when seen side by side. The average North African ostrich is a much taller bird than the South African, being longer in the legs and neck. The head reaches to a height of from eight to nine feet from the ground whereas in the Cape bird it extends only seven to eight feet. The feet, legs and neck of the Nigerian bird are also more robust. The general dimensions of the body do not differ much in the two, the greater size of the northern being mainly a result of the longer legs and neck. As a chick and young bird the body of the northern ostrich however tends to narrow behind more than the southern, but later this becomes a feature largely dependent upon the nutritive state. The relative sizes admit of the two being easily picked out in a mixed troop, the heads of the northern birds towering a foot or so above those of the southern.

The cross-bred birds at maturity stand higher than pure Cape birds, but are not so high as the Nigerian. As chicks the body tends to narrow behind more than in Cape chicks, so that, with the slightly longer legs and tapering body, they appear decidedly more slender than Cape chicks of the same age. On the whole it can be said that as regards size, especially length of limbs and neck, the hybrids follow neither one parent nor the other but are intermediate between the two, though the statement is not one which can be supported by actual measurements.

The two chicks of the second cross-bred generation already reared are now a year old and as regards their general size are strikingly like the South African grandparents as contrasted with the North African, including the shorter, less robust legs and neck. When mingling with first generation cross-breds of the same age the difference is most marked, and no one would hesitate in regarding them as pure Cape birds. Such a result is at least suggestive that the distinctive sizes of the northern and southern ostrich will undergo segregation in the F_2 generation, but further chicks will be necessary before the real nature of the segregation can be determined.

COLOUR.

The skin or body colours of the ostrich, as distinct from those of the plumage, vary from the chick to the adult stage, are different in the hen and the cock, and change in the latter with the breeding state. They also vary with the physiological condition of the bird, according as the surface of the skin is clean or covered with scurf. When low in condition the skin becomes dry and scaly, thereby masking the true colours; but as a higher physiological state is reached the scurf peels off or is preened off, and the true fresh colour is revealed. This is particularly the case at the beginning of the breeding season when the skin colours are at their brightest. The colour is readily seen on the naked legs and under the wings, around the eyes and beak, and elsewhere by turning aside the overlapping feathers. Chicks of both sexes are practically alike and even young birds show little distinction. The hen remains throughout of the same colour as young birds, but the cock undergoes a change beyond, and in places assumes a brilliant scarlet as the nuptial state is attained. From the chick onwards the colour distinctions between the North African and South African ostriches are strongly marked.

The red and scarlet colouration of the cocks of both races, as well as the rich dark blue of the Cape bird, are found to be dependent upon the presence of the testes, while the black plumage is dependent upon the absence of the ovaries. South African cocks which have been castrated while young never assume the red and scarlet skin colours, but retain the light or dark grey of all young birds and mature hens. On the other hand the plumage of castrated cocks attains the normal blackness of the sex as contrasted with the greyness of the hens, from which it may be inferred that the formation of the black pigment of the feathers is not subject to any influence from the male gonads. Spayed hens retain their ordinary body colour but the normally grey feathers are found to assume the blackness of the cock, showing that ordinarily the secretions from the ovaries exercise an inhibitory influence on the formation of black pigment in the feathers of the hen, though having no action on the skin colour¹.

¹ Prof. T. H. Morgan, *Amer. Nat.* Vol. LI. Sept. 1917, in the case of the cock bird of the Sebright bantam which is "hen-feathered," has proved experimentally that when castrated a complete change in the plumage occurs, normal cock feathers appearing. He considers that, as in the hen, some internal secretion, acting through the gonad, must inhibit the development of the secondary sexual characters in the hen-feathered cock. Morgan also refers to certain experiments by Goodale who has found that when the hen of

The secondary sexual colours of the skin and plumage of the ostrich are thus determined by altogether different influences; the full attainment of the one is dependent upon the presence of the testes and of the other upon the absence of the ovaries. Two North African birds at Grootfontein, although about six years old, have shown no signs of sexual maturity; they retain the cream yellow of all northern young birds and mature hens, but have the black plumage of cocks. Evidently some abnormality is connected with the internal gonads, but from the external appearance of the birds it is impossible to say whether they are cocks or hens. It may be noted that the removal of the ovaries or testes, especially after a bird has attained maturity, has little or no effect on certain of the sexual instincts. Thus a castrated hen will go through the characteristic snapping of the beak and fluttering of the wings as if broody, and will even crouch to receive the cock; while the castrated cock will perform his ordinary "rolling" display and even mount a crouching hen.

In determining the sexual colours of the male ostrich the testes clearly give rise to some secretion, presumably of an enzyme nature. This must be produced at first in small quantities, and the colour changes come slowly; but as the testes ripen and become functional more of the enzyme must be forthcoming, for the colour intensity increases and remains brilliant throughout the mating period. With the beginning of the six weeks' period of incubation the testes become less active, pairing ceases, less enzyme is produced and the colour fades. The differences between the sexual colours of the northern and southern bird are well defined, and must be germinal in the first instance; but the factors must act through the gonads, and presumably these exert their influence by means of specific enzymes. Even if we regard the germinal factors as themselves enzymes, as Troland¹ and others would have us do, those concerned with the sexual colours must express themselves through the gonads.

Though the scarlet colour of the cock is a secondary sexual character it may well be doubted whether it has any influence on the mating of the birds, or any preferential value in the eyes of the hen, as is so often

ordinary breeds of fowls is spayed she develops the full male plumage, as is also proved above for the hen ostrich. Seeing that the plumage of the cock ostrich is more valuable than that of the hen the results from spaying the latter have an economic bearing and the practice is followed by some farmers.

¹ Troland, L. T., "Biological Enigmas and the Theory of Enzyme Action," *Amer. Nat.* Vol. LI, June, 1917.

supposed to be the case with the bright nuptial colours of birds. For northern cocks are a bright scarlet over all their exposed parts at the time of sexual ripeness while southern cocks are scarlet only over the head and the tarsus and are far less striking in their general appearance, yet a northern hen will crouch just as freely for the latter as for the former. Occasionally ostriches exhibit a dim suggestion of preferential mating, but in practice it is found that any hen will pair with any cock, and in "camping off" as breeders the farmer never takes into account any possible preferences on the part of the birds themselves. In a state of nature, on the open veld, a cock gathers round him one or more hens as the breeding season approaches, and very definite spatial limitations become established among the different breeding sets, and woe betide any cock which may wander on the area appropriated by another. In all this however the hens are purely passive and indifferent, and are prone to lay in the same nest, as many as 60 or 70 eggs being sometimes found in the one shallow depression. Further, as in most other birds, the plumage is at its highest state of development at the beginning of the mating season, as if still further adding to the attractiveness of the cock. Yet farmers as often as not clip the plumes before mating birds, and so preserve them from wear and tear, without however any influence on the readiness with which pairing takes place.

The Northern Ostrich. In North African chicks the skin is a bright deep yellow, almost orange, over the legs and head, and a slightly paler yellow over the body and neck. As maturity is reached the hen becomes a light yellow, the tarsal scales assuming a light or dark horny brown. Some northern hens are slightly pink over parts of the body, and the colour may show through the white downy covering of the neck.

The North African cock undergoes remarkable colour changes as sexual maturity is attained, which are a sure guide to the farmer as to the breeding condition of the bird. The deep yellow of the chick is gradually replaced by a light yellow, this by pink, and then by red, reaching a bright scarlet over the legs, body, neck and head as the actual mating period is reached. The bright scarlet colour contrasts strongly with the jet black body-feathers, white waving wings, erect light brown tail feathers and fleecy white down of the neck, and makes of him a glorious creature as he prances about in his breeding camp in all the pride and pugnacity of his sex. The nuptial colours pale greatly when nesting begins, and also when the breeding season is over, the body being reduced to a pale pink or brick colour. At its height so

sensitive is the colour to the physiological state of the bird that close observation often reveals variations in the intensity within the same day, as well as from day to day.

The Southern Ostrich. The skin of South African chicks is at first pale yellow in colour and afterwards dark grey. Highly fed Cape chicks may show a rich deep yellow round the eyes and beak, though this does not continue for more than a few months. Mature southern hens are a dark grey over the legs, body, neck and tarsal scales.

The Cape cock is at first a dark grey or steel colour, much like the hen, but as sexual maturity is gained he assumes a fresh, bright blue over the greater part of the body, while the tarsal scales, beak and naked parts round the eyes become a bright scarlet; the small scales over the sides and hind part of the tarsus may also be red or pink, but ordinarily none of the red colouration extends beyond the tarsus, nor over the body and neck.

Thus northern chicks are a deeper yellow than southern chicks. They pass to a pale yellow and the hen remains at this stage, but the cock passes beyond to a pink and then a scarlet stage. The pale yellow of southern chicks is early replaced by a dark grey which persists in the hen, but is followed in the cock by a blue or blue grey as sexual maturity is reached; moreover, only the tarsal scales, beak, and skin round the eyes assume the bright scarlet which characterises practically the entire body of the northern bird.

In southern cocks the red colour of the northern would appear to be latent, or perhaps wholly obscured by the dark blue; for on recovering from an injury to the neck or body it is often found that the scar of the new skin shows a reddish tinge.

Cross-bred Ostriches. The colour of the skin of cross-bred chicks is intermediate between that of northern and southern chicks. The legs, body and large scales are a pale yellow, which is lighter than that of Cape chicks but never so deep as that of Nigerian chicks. The adult cross-bred hen retains the light yellow body colour, though usually it becomes a little darker compared with the chick. The colour remains darker than that of the pure northern hen, but is invariably lighter than the pure Cape.

The cross-bred cock retains the uniform light yellow of the hen until sexual maturity approaches. He then assumes a pink tinge in places and later the bright scarlet. As noted, however, it is in the

extent of the red colouration, not in its intensity, that the northern and southern cocks differ so conspicuously; in the former it is diffused practically all over, while in the latter it is limited to the head and legs below the ankle joint. The sexually mature cross-bred cock is decidedly intermediate between these two as regards the area of the body assuming the red colour. The head and tarsus are scarlet as in both parents, but only a slight pink colour appears on the upper part of the leg and also over the neck, and may even tinge the other parts of the body, though without approaching the bright red of the northern parent. The various cross-bred cocks naturally differ as regards the degree and extent of the colouration, but they never wholly follow one parent or the other. In extreme cases the body colour may be a grey blue almost like the southern cock or a grey yellow nearly like the northern, but all kinds of intermediate tints are to be met with, even in birds from the same nest.

In all the cross-bred cocks the red of the neck is displayed to a greater or less degree through the white downy covering. Sometimes it is only apparent when the small hairy feathers are turned aside and the loose skin put on the stretch. It then appears as red showing through a bluish ground, the two producing a purple. From this it would seem as if both the blue of the southern bird and the red of the northern were represented, the resultant purple being the product of the two. It is evident that the degree of redness of the body and neck is partly dependent upon the other body colours. If the latter is dark blue it naturally tends to obscure the red, while if the body colour is only a pale yellow the red becomes more obvious. The nuptial colours, dependent upon the presence of the testes, are superimposed upon the true body colours.

As regards the two F_2 chicks the colour of the body, legs and neck is quite as dark as that of any Cape hen, showing no influence from the lighter colour of the northern grandparent and the intermediate light colour of the cross-bred parents. Both being hens however the colour is not so distinctive as it would be in the case of cocks. Taken as a preliminary result it certainly suggests that the colours of the northern and southern birds have a separate factorial basis, and that segregation will take place in the second cross-bred generation. The rearing of further F_2 chicks will be awaited with interest as likely to solve the problem.

BALD HEAD PATCH.

The crown of the head of the South African ostrich is covered with short, hair-like feathers, which often form a tuft of longer hairs in the middle. A bare pineal spot¹, present in all ostriches at the back of the head, is so small in the adult as to be only noticeable when the feathers are turned aside. The North African ostrich on the other hand is distinguished by having the top of the head for the most part naked, a bald patch beginning at the back and extending forwards in a shield-like fashion between the eyes (Text-fig. 1, p. 158). The area is roughly pear-shaped, but may be partly divided down the middle. In diagnostic descriptions the baldness is considered to be a character of some importance in separating the northern species from the southern and is even mentioned in the writings of Pliny².

The extent and shape of the naked space vary a little in different ostriches, but all the North African birds at Grootfontein display it to a greater or less degree. It is quite independent of the pineal spot, and its posterior border may either include this (Text-fig. 1) or pass in front of it. In some birds, instead of forming a continuous patch, it is divided more or less down the middle, having then a decided bilaterality. Often a tuft of long, hair-like feathers remains towards the middle of the hinder border, corresponding with the tuft in the southern bird, and gradually disappears forwards. The area is covered with a horny, scurfy layer, which peels off at times, exposing a fresh, clean surface of the skin with the hard bony skull immediately below.

The baldness is not apparent in the North African ostrich chick when first hatched. At that time the head is covered with short bristly down as in the South African, and the character becomes established in the course of the first six months or so of growth. It is gradually formed by the dropping out of the hairy feathers from about two months onwards, and in a batch of chicks of the same age practically all stages in the loss can be observed, the feathers to remain longest being those of the middle tuft. No sharp line of separation occurs between the naked and the covered part of the head; a few stunted feathers represent

¹ An extra-cranial pineal body has lately been discovered in the ostrich. At a certain stage of development it shows as a black pigmented area or vesicle which later disappears, and only a dark, oval area, devoid of feathers remains in the newly hatched chick and persists throughout life. Apparently the ostrich is the only bird with such a well-defined pineal body, recalling that of the reptilia and persisting as a pineal spot.

² *Hist. Mund.* lib. xi. cap. xxxvii.

the gradual transition, while the medium tuft may or may not persist. No corresponding falling out of the feathers ever takes place in the Cape chick.

Naturally some interest has been attached to the behaviour of the bald patch in the crosses of the northern and southern ostrich. Of the hundred or so cross-bred chicks which have been hatched none at first showed any signs of baldness, but in every case the feathers began to fall out when the chick was two or three months old, and at six months the patch was established as completely as in adult North African ostriches. Thus the baldness of the northern bird is shown to be dominant over its absence in the southern bird.

The two second generation chicks already reared are now well over the age at which the bald patch becomes established, and in one of them the head remains covered with hairy feathers as in southern birds while in the other the baldness has been formed as distinctly as in any northern bird. The F_2 chicks thus afford evidence that factorial segregation takes place in the second hybrid generation, and there can be little question that when sufficient chicks of this generation have been obtained it will be found that baldness behaves as a homozygous dominant in strictly Mendelian proportions.

The bald head patch is therefore a distinctive Mendelian unit-character separating the northern and the southern ostrich. The differences associated with the dimensions and colours of the birds, and also those of the egg, are differences of features common to both, but in the Cape bird there is nothing suggestive of the baldness of the Nigerian. It is an entirely new character which has appeared in the latter race of ostriches, but not in the former. It may be regarded as a mutation, and was presumably fully developed from the beginning, for though it varies somewhat in its extent and form the differences are no more than can be regarded as fluctuating variations. That it is germinal in its origin is manifest since it appears in all chicks, both pure and cross-bred, while its dominance in all the latter proves that the parents are duplex or homozygous with regard to it.

It can hardly be supposed that the baldness has arisen in response to any external influence, for it is unlikely that anything environmental could affect the top of the head of the northern bird which would not have a corresponding action upon its southern relative, even if it were possible that any influencing of the kind could bring about a corresponding change in the germ plasm. Nor can it be deemed to have any adaptive value. It lends strong support to the view maintained by

Bateson, and also by Morgan, that new characters make their appearance as a result of changes in the germ plasm, without any reference to external influences, or any utilitarian value or need of the individual. Since the baldness is now present as a duplex dominant in all the imported birds it must have originated long ago in the history of the northern ostrich, sufficiently long for the change to have affected all the individuals. For, as will be shown later, there is good reason to suppose that in the ostrich a new character appears at first in only a few members, but gradually extends to more and more, by the continued change *de novo* in the germ plasm of the nulliplex members of the race.

THE EGG.

As in all other birds the eggs from the same ostrich and also from different ostriches vary within certain limits, as regards size, shape and surface characters. Beyond these fluctuating variations however certain well-defined differences distinguish the egg of the North African from that of the South African bird (Pl. VII, fig. 3).

Egg of North African Ostrich. The egg of the northern bird is practically always larger than that of the southern, the shell is almost free from obvious pores or pittings, and presents an ivory-like smooth surface. Usually also the northern egg is rounder in shape or less oval. Measurements have been taken at the nest of the long and short diameters of four series of eggs and are as follows, in inches:

TABLE I.

Measurements of Eggs of North African Ostrich.

Series A			Series B		
	Long Diameter	Short Diameter		Long Diameter	Short Diameter
1	6.00	5.19	1	6.12	5.50
2	5.94	5.19	2	6.50	5.50
3	6.00	5.25	3	6.12	5.38
4	6.12	5.31	4	6.38	5.25
5	6.19	5.31	5	6.12	5.25
6	6.19	5.31	6	6.25	5.38
7	6.12	5.31	7	6.38	5.25
8	6.00	5.25	8	6.25	5.38
9	6.00	5.25	9	6.00	5.50
10	6.00	5.19	10	6.25	5.50
11	5.94	5.19	11	6.25	5.38
Average	6.05	5.25		6.24	5.43

TABLE 1—*continued*.

Series C			Series D		
	Long Diameter	Short Diameter		Long Diameter	Short Diameter
1	6.25	5.38	1	6.06	5.38
2	6.25	5.31	2	6.31	5.38
3	5.94	5.12	3	6.25	5.38
4	6.00	5.19	4	6.06	5.44
5	6.12	5.44	5	6.12	5.31
6	6.25	5.50	6	6.25	5.31
7	6.25	5.38	7	6.25	5.38
8	6.06	5.25	Average	6.19	5.37
9	6.12	5.38			
10	6.25	5.44			
11	6.00	5.19			
12	6.12	5.38			
13	6.06	5.31			
14	6.25	5.50			
Average	6.14	5.34			

Thus the average long diameter of 43 northern eggs is 6.15 inches and the short diameter 5.35 inches, representing an average difference between the two axes of 0.8 inch.

Egg of South African Ostrich. The egg of the southern bird is deeply pitted all over the surface, and pits often larger and more plentiful at the air-chamber end, hence the shell does not present the ivory smoothness of the northern egg. A Cape hen will sometimes produce a nearly smooth, round egg, but never to so marked a degree as the typical Nigerian hen. Also the latter may occasionally lay eggs with deeper pittings than usual, especially in the first one of the season. Among a number of eggs from northern and southern birds mixed together no mistake can however be made in separating the one type from the other.

The pitting which gives such a marked character to the southern egg is associated with the respiratory pores of the shell. In the northern shell the pores are so small and open so close to the surface as to be scarcely visible to the naked eye, and are mostly scattered singly with but little grouping. Hence the surface appears almost uniformly smooth, though fine pores can be detected with a lens. In the southern egg the shell pores are larger, sunken below the general surface and mostly in small groups, varying from about six to twelve in a group. It is the close grouping of the sunken pores which gives rise to the pitted surface. In eggs which have been in the nest for some time dirt tends to accumulate within the pits and thus accentuates their presence, whereas in the northern egg the pores are too small and shallow. In both types the

outer enamel layer shows differences in thickness and with it the polished character of the surface. All the eggs are a cream or yellow colour when freshly laid but fade considerably on exposure.

Measurements have been taken of 20 eggs as follows:

TABLE II.

Measurements of Eggs of South African Ostrich.

	Series A			Series B	
	Long Diameter	Short Diameter		Long Diameter	Short Diameter
1	6.00	4.62	1	6.25	5.12
2	5.69	4.81	2	5.81	4.88
3	6.00	5.12	3	5.75	4.94
4	5.62	4.88	4	5.94	5.06
5	6.25	4.81	5	6.00	5.00
6	5.69	4.50	6	5.81	5.00
7	6.12	4.81	7	6.00	5.00
8	5.81	5.00	8	5.88	5.00
9	6.06	5.00	9	6.00	5.00
10	6.00	5.00	10	5.94	5.06
Average	5.92	4.85		5.93	5.00

Thus the average long diameter is 5.92 inches and the short diameter 4.92 giving a difference of 1 inch. With such variable structures as eggs a larger series of measurements is desirable in order to secure a more reliable comparison. They serve to show however that on the average the northern egg is about a quarter of an inch longer ($6.15 - 5.92 = 0.23$ inch) and two-fifths of an inch broader than the southern egg ($5.35 - 4.92 = 0.43$). The mean difference in the two diameters is 0.8 inch for the northern and 1 inch for the southern, indicating that the former are rounder or less oval than the latter.

Eggs from Cross-matings. In breeding for cross-bred chicks the eggs follow the characteristics of the hen whatever the cock may be, that is, the eggs laid by a northern hen mated with a southern cock are large, round and unpitted, while those from a southern hen mated with a northern cock are smaller, oval and pitted. Thus as regards size, shape and surface features, the egg as laid is uninfluenced by the male bird and partakes wholly of the nature of the hen. This is what would ordinarily be expected, seeing that the germ from the cock unites only with the germ of the hen, and scarcely any further change takes place before the egg is laid. As the albumen, shell-membranes and shell are formed in the oviduct of the hen after fertilisation it is difficult to see how the coverings of the egg could be influenced. Instances are adduced however where in crosses of other birds giving differently coloured eggs

the cock seems to exercise some influence, the phenomenon being spoken of as *Xenia* (*Journ. Heredity*, Vol. VI, No. 5). The diverse characters of the eggs of the northern and southern ostrich afford a good test case of the possibility of *Xenia* occurring, but from none of the cross-matings has any indication of the phenomenon been forthcoming.

Eggs from Cross-bred Hens. In cross-bred hens are naturally combined the possibilities of both the northern and the southern parents, and the characters of the eggs laid by them are just as much a part of the make-up of the bird as are the more obvious body features. The question therefore arises whether the eggs laid by cross-bred hens will follow those of one parent or the other, or be something intermediate between the two. Scores of eggs laid by cross-bred hens have been examined at the nest and in the incubator and in all cases have been found to be

TABLE III.

Measurements of Eggs of Cross-bred Ostriches.

Series A			Series B		
	Long Diameter	Short Diameter		Long Diameter	Short Diameter
1	6.19	5.12	1	6.25	5.12
2	6.25	5.12	2	5.81	4.94
3	6.12	5.19	3	5.94	4.94
4	6.12	5.06	4	6.00	5.00
5	6.12	5.12	5	6.19	5.12
6	6.12	5.06	6	5.88	4.94
7	6.06	5.12	7	6.25	5.12
8	6.00	5.00	8	6.19	5.06
9	5.88	5.00	9	6.19	5.06
10	5.94	5.00	10	5.88	5.00
Average	6.08	5.08		6.06	5.02

Series C

	Long Diameter	Short Diameter
1	5.94	5.12
2	5.81	5.12
3	5.88	5.12
4	5.75	5.06
5	6.00	5.12
6	6.06	5.06
7	5.62	5.12
8	5.94	5.12
9	5.88	5.06
10	5.88	5.12
11	5.81	5.12
12	5.88	5.00
13	6.06	5.12
14	5.94	5.12
Average	5.89	5.09

intermediate as regards size, shape and the nature of the shell between typical northern and southern eggs. Obvious pittings occur over the shell, often more numerous towards the air-chamber end, but are never so plentiful nor so deep as in the eggs from the southern bird. The egg has neither the full size nor the roundness of the northern ostrich, but is larger than the southern, and its general surface is more enamel-like. Naturally variations occur in the eggs laid by different hens, and sometimes they approach those of the one parent more nearly and sometimes those of the other. The degree of pitting and smoothness of the surface of the shell do not admit of more than a general statement, but the dimensions of 34 cross-bred eggs are available for comparison with those of the northern and the southern eggs.

The average long diameter of 34 cross-bred eggs is therefore 6·01 inches and short diameter 5·06 inches, the difference between the two diameters being 0·95.

The measurements of the three series may be compared as follows :

	Long Diameter	Short Diameter	Difference
43 North African Eggs ...	6·15	5·35	0·80
34 Cross-bred Eggs ...	6·01	5·06	0·95
20 South African Eggs ...	5·92	4·92	1·00

Though not elaborate enough for many purposes the results suffice to indicate that as regards size and shape the cross-bred eggs are intermediate between those of the northern and the southern bird. They apparently approach somewhat nearer to the southern than the northern, but with such variable objects as eggs an indication of this kind may be deemed of little value when only small numbers are available.

The intermediate nature of the cross-bred eggs, as regards size, shape and the nature of the surface, may be taken to suggest that the different characteristics of the eggs of the two races of ostrich are dependent upon separate factorial representation in the germ plasm, as in the case of the dimensions and colours of the birds. Also the factors are not alternatives, for in the hybrid egg no one character of the parents is dominant or recessive to the other, but each strives, as it were, for expression, the result being something midway between the two.

THE WING QUILLS.

In farming practice the number of plumes to the wing of the ostrich is an important matter, though selective breeding has hitherto been concerned with the quality of the plumes rather than with their quantity, it having been assumed that not much variation in number occurs. The

first row of plumes, the wing quills or remiges, includes the familiar white plumes of the ostrich which are by far the most valuable as compared with the first and second rows of upper-coverts which are also clipped, and are black in the cock and grey in the hen. As the coverts alternate with the wing quills the numbers in all the rows are definitely correlated, so that for purposes of comparison among different birds attention can be confined to the first row, the remiges (Pl. VII, figs. 1 and 2)

North African Ostriches. The first-row feathers on each wing have been counted on 25 of the original imported North African birds and the results are given below. It will be noted that a difference of one or two plumes is often found between one wing and the other, but the cocks and hens show no distinction. The number on the wing varies from 33 to 39, the arithmetical mean of the series being 36.54; represented graphically they approximate to a normal frequency curve with the mode at 36. Manifestly the birds represent a mixed population, a result of indiscriminate breeding in a race in which the numbers differ by small amounts; but indications are not wanting that a pure line can be built up of each number. We may regard each bird as heterozygous with regard to number of plumes, and a mixture of the kind given below is what would be expected seeing that the birds come from a single area in North Africa where no farming selection is practised.

TABLE IV.

First-row Plumes on Wings of Imported North African Ostriches.

	Right Wing	Left Wing		Right Wing	Left Wing
1 Hen, No. 11	39	38	14 Hen, No. 87	36	37
2 Hen, No. 20	37	37	15 Cock, No. 92	37	36
3 Hen, No. 40	36	37	16 Hen, No. 105	36	37
4 Hen, No. 41	34	35	17 Hen, No. 108	37	36
5 Hen, No. 45	36	36	18 Cock, No. 115	38	38
6 Cock, No. 50	36	37	19 Hen, No. 116	33	34
7 Hen, No. 63	37	38	20 Hen, No. 130	35	36
8 Hen, No. 69	38	39	21 Cock, No. 141	35	34
9 Hen, No. 71	36	35	22 Cock, No. 252	38	39
10 Hen, No. 75	37	36	23 Hen, No. 277	35	36
11 Cock, No. 78	37	36	24 Cock, No. 284	36	37
12 Cock, No. 84	35	36	25 Hen, No. 287	38	39
13 Cock, No. 85	38	38			

The number of plumes on the wings of 15 pure North African chicks reared at Grootfontein from the importation, are also represented and give approximately the same arithmetical mean as the above, namely 36.7, though without the low numbers 33 and 34. The chicks are from

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three separate breeding sets and may represent some slight selective influence compared with the mixed importation.

TABLE V.

First-row Plumes on North African Ostrich Chicks reared at Grootfontein.

		Right Wing	Left Wing			Right Wing	Left Wing
1	No. 333	36	36	9	No. 6	36	37
2	No. 334	36	37	10	No. 7	39	38
3	No. 335	37	37	11	No. 8	38	37
4	No. 1	37	37	12	No. 9	35	36
5	No. 2	38	38	13	No. 10	38	37
6	No. 3	36	36	14	No. 11	36	38
7	No. 4	36	36	15	No. 12	36	35
8	No. 5	36	36				

South African Ostriches. In order to ascertain the number of plumes on the South African ostrich for comparison with the North African recourse has been had to the troops on various ostrich farms. Included among them are representatives from the best and most widely known ostrich strains of South Africa. It is only necessary to give the detailed countings of one series of 48 birds as an example, the average for the others agreeing closely. As much interchange of birds and chicks has taken place since ostrich farming commenced it is most unlikely that any additions to the series will vary from the averages here given.

TABLE VI.

Number of Plumes on South African Ostriches. Farmers' Series. No. 4.

		Right Wing	Left Wing			Right Wing	Left Wing
1	Hen	39	39	26	Cock	39	39
2	Cock	35	35	27	Cock	36	35
4	Cock	37	36	29	Cock	39	39
5	Cock	36	37	30	Cock	38	38
6	Hen	36	36	31	Hen	37	34
7	Cock	37	36	32	Hen	37	38
8	Hen	37	36	33	Hen	36	35
9	Cock	37	38	34	Hen	36	37
10	Cock	36	36	35	Cock	36	36
11	Cock	36	35	36	Cock	36	35
12	Cock	37	37	37	Cock	35	36
13	Hen	39	38	38	Hen	35	35
14	Cock	36	36	39	Hen	37	38
15	Cock	38	38	40	Cock	36	35
16	Hen	36	36	41	Cock	39	38
17	Cock	38	37	42	Cock	39	38
18	Cock	36	37	43	Cock	40	39
19	Cock	37	36	44	Cock	35	34
20	Cock	38	37	45	Hen	37	38
21	Cock	39	38	46	Cock	36	36
22	Hen	34	34	47	Hen	37	36
23	Hen	38	38	48	Hen	37	36
24	Hen	37	36	49	Cock	37	37
25	Cock	39	40	50	Cock	39	38

The arithmetical means of five Farmers' Series are as follows:

No. 1	25 birds	36.88
No. 2	24 birds	36.58
No. 3	19 birds	36.97
No. 4	48 birds	36.87
No. 5	19 birds	36.63
Total Mean		36.78

The average number of plumes on the South African ostrich is therefore the same as that on the North African, an important conclusion which could not have been arrived at without the opportunity of counting large numbers of each.

As the northern ostriches now at Grootfontein were all procured originally by the Arabs of Nigeria as chicks from wild nests, and are uninfluenced by any artificial breeding, we may presume that their plumes represent the average for the North African wild bird, and we have therefore good reason for concluding that the ostriches over the whole continent of Africa produce the same average number. From this it follows that *during the fifty years of ostrich farming in South Africa no advance has been made on the number of plumes originally present on the wild bird*. On the average the domesticated birds of to-day produce the same quantity of plumes as the original birds with which the first ostrich farmers commenced in the sixties.

Though somewhat remarkable at first sight this result is scarcely to be wondered at if we bear in mind the principles underlying ostrich breeding: *Farmers have bred for quality; quantity has never been taken into account*. Great advances have been made in the so-called quality characters of the individual plume, but in doing this no attention has been given to the number of feathers which one bird produces as compared with another, and therefore no numerical change has taken place. It is a good instance of the principle that no progress is ever made as a result of indiscriminate breeding, unless a character has some selection value, or mutations are taking place.

Cross-bred Ostriches. Seeing that the northern and southern birds have the same average number of plumes and are a mixture of heterozygotes, no change is to be expected in the number of plumes on cross-bred chicks compared with what would be procured by mating two northern or two southern birds. The table given below is an example of the results which have been obtained. The arithmetical mean of the parents is 36.24 and of the chicks 36.28, but for a larger series the average is 36.31 which agrees more closely with that of the two races.

A hint at factorial purity is indicated seeing that the extremes 33, 34 and 39 are not represented.

TABLE VII.

Number of first-row Plumes on Cross-bred Chicks from mating a North African cock with a South African hen.

Parents :				Right Wing	Left Wing
North African cock, No. 78				37	36
South African hen, No. 225				36	36
Chicks :					
1	No. 300	36	36
2	No. 301	35	36
3	No. 315	38	37
4	No. 316	36	37
5	No. 318	37	36
6	No. 320	35	36
7	No. 321	36	36
8	No. 322	36	35
9	No. 323	38	37

Survival of 42-plumed Ostriches. Among the Cape birds in the Grootfontein flock two¹ have been discovered with 42 plumes to the first row, though the rest have the usual average of about 36. At first it seemed as if two distinct strains of ostriches were represented in South Africa, as compared with the single strain in North Africa, one with approximately 36 plumes as the average and another with 42. The countings on farmers' birds have however given no support for this view; they have disclosed no individual bird exceeding 40 plumes, nor any influence from a 42-plumed strain. Hence it is concluded that the occurrence of ostriches with 42 plumes is altogether exceptional among Cape birds, and has had no recent influence on the general average. Likewise none of the Nigerian birds has more than 39 plumes, nor any of the chicks reared from them; so the influence of a 42-plumed strain is non-existent in North Africa.

As regards their origin it is manifest that the 42-plumed birds represent a distinct departure from the ordinary 36-plumed birds. Were no other evidence available the high number might be looked upon as a meristic mutation and, as will be proved later, the birds give progeny with such high numbers as to show that the extra plumes are not merely the extreme limit of a fluctuating series but have a factorial value. They

¹ Of the two original birds one has since met with an accident and died. The birds were procured several years ago from two farmers widely apart, without any suspicion of their number of plumes. It is noteworthy that though search had since been made among the same flock yet in neither case has another 42-plumed bird been found.

have also been considered in the light of reversions to an earlier ancestry, but fuller consideration leads us to account for them otherwise. Recent observations, to be fully described in a later paper, have shown that the ostrich presents us with numerous stages indicating the course of the degeneration which the wings and legs have undergone up to the present, as well as the course likely to be followed in the future. Survivals of many ancestral characteristics are to be found among the plentiful material now available for study. Thus, while ordinarily only one incomplete row of under-coverts is present, a farmer's strain exists in which a second row of under-coverts is almost complete, and several members of a third row also occur, and single members of both rows are occasionally met with on other birds. All stages are to be found from a complete row of under-coverts to the usual one where 8 to 10 are wanting at one end of the row (Pl. VII. fig. 2); and conditions of a like nature are to be met with in the second row of upper-coverts. Vestigial down is to be found on most ostriches over the wings and tail, though it is usually stated to be absent. Further, while usually buried in the flesh of the wing, the third digit sometimes bears a second phalanx and projects freely from the surface, and even bears its own plumes, a primitive condition suggestive of the fossil bird *Archaeopteryx*. The claw is usually absent from the small, fourth toe of the foot but still survives in a few; while the scales on the middle toe show the beginnings of loss by one or two breaks in their continuity (Text-fig. 2, p. 182). Experiments hitherto carried out all indicate that the individual losses have proceeded as retrogressive mutations, on definite factorial lines and in well-defined, determinate directions.

In view of all these survivals of many of the earlier characteristics of the ostrich the 42-plumed bird may with good reason be regarded as a survival of a stage when the average number of plumes to the wing was larger than at present. On this interpretation the 36-plumed birds of to-day are to be considered as degenerate in the number of wing quills, as they are in many other respects. The practical endeavour is now being made to build up a pure strain of ostriches bearing 42 plumes, for with the increase of the other rows of plumes in correlation with the wing quills it becomes possible to provide the farmer with an ostrich giving about $25\frac{1}{2}$ more plumes than he receives from his birds at present, the "quality points" being also of the highest. The demonstration below that the high number is not merely the extreme of a fluctuating series, but is factorial in its nature, renders this possible. Whether by continued selection the number 42 will ever be exceeded

is doubtful, seeing that the factors for any higher number have probably been altogether lost to the race, even if they were ever present in the ancestral ostrich.

Table VIII shows that when the 42-plumed southern cock is mated with various North African hens of the 36 strain the average number of plumes in the progeny is practically intermediate, namely 39.56, the lowest number being 37 and the highest 42; they do not regress to the general average. The numbers form an approximately normal curve with the mode at 40. None of the birds hitherto employed as breeders can be deemed "pure" as regards the number of plumes; and the 42-plumed bird is probably heterozygous like the rest; hence the fluctuating series represented below.

TABLE VIII.

*Number of first-row Plumes on Cross-bred Chicks from mating a
42-plumed Southern cock and 36-plumed Northern hens.*

		Right Wing	Left Wing			Right Wing	Left Wing
1	No. 226	42	41	13	No. 311	40	40
2	No. 228	39	40	14	No. 230	41	40
3	No. 229	41	42	15	No. 232	39	41
4	No. 242	40	40	16	No. 233	41	42
5	No. 243	39	40	17	No. 234	42	40
6	No. 302	37	38	18	No. 237	40	39
7	No. 303	38	38	19	No. 238	39	38
8	No. 304	40	40	20	No. 1	39	38
9	No. 307	38	38	21	No. 2	39	39
10	No. 308	40	39	22	No. 3	38	39
11	No. 309	37	39	23	No. 4	40	41
12	No. 310	38	39	24	No. 5	41	40

Experiments are being undertaken to determine how far it is possible to extract numerically pure lines, especially as regards the two extremes 33 and 42, but progress is necessarily slow. Until this has been done full proof will be lacking that each plume has its own factorial representation, though all the evidence points in this direction.

The 33-plumed birds represent the extreme of the loss of wing quills which has taken place in the ostrich of to-day compared with the maximum of 42 plumes. If, as we seem bound to suppose, some intrinsic influence is at work within the germ plasm inducing slow retrogressive changes, it appears not unlikely that by in-breeding pure 33-plumed birds it will be possible to increase the action of the degenerative force and produce a still further loss of plumes. By selection it should be possible to control the further evolution of the ostrich with regard to the number of its plumes.

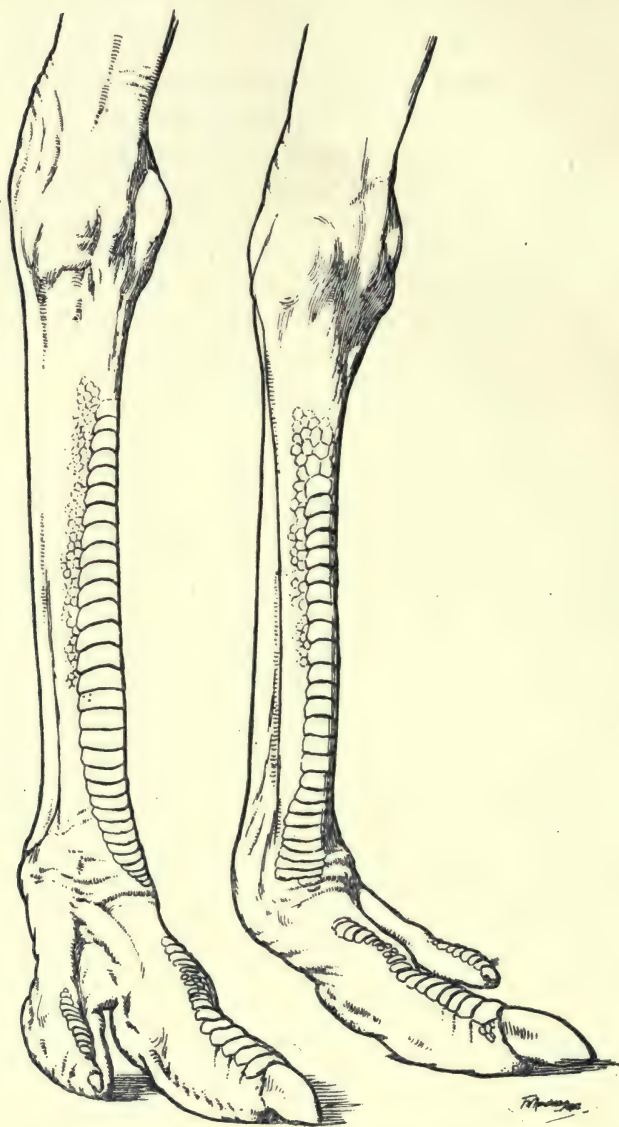
As noted earlier, farming practice has established as clearly as could be expected that the different "points" of the plumes are factorial in their behaviour, and as they vary in the various strains a separate germinal representation for each may be assumed. Even such a simple structural part as the central shaft of the feather shows many different types which appear either distinct or as intermediates in crosses. The length of the barbules and their closeness on the barbs are also matters of much economic importance in determining the "density" of the plumes, and the farmer never questions their distinctness in breeding. At the present stage of the ostrich industry, where crossing is practised to such a high degree, the factorial analysis of the individual plume would be a prolonged undertaking, but will become feasible as the farmer finds himself in a position to build up pure strains.

SCUTELLATION OF MIDDLE TOE.

Along the front of the tarsus extends a series of large, nearly rectangular scales, which in most cases continues uninterruptedly to the end of the big middle toe. Over the leg the contiguous edges of the scales simply meet, but they are imbricated where the tarsus joins the toe and also over the latter, thus allowing for the bending movements of the toe during walking and running. Along the tarsus the scales retain approximately the same size, but at the junction with the toe they usually become somewhat smaller, enlarging again distally. Occasionally a distinct break in the continuity occurs at the joint, several of the large scales disappearing and being replaced by insignificant ones like those which cover the surface of the limb generally; and in rare cases a second break in the continuity takes place over the joint about the middle of the toe, thus giving a proximal and distal series of digital scales (Text-fig. 2, p. 182).

The number of scales fluctuates in different individuals, and also on one limb as compared with the other; and occasionally irregularities are introduced owing to single scales being partly subdivided. At the breaks the large scales tend to pass insensibly into the small scales of the limb, hence any enumeration is only approximate. A few countings are given in Table VIII, p. 183.

The break in the continuity occurs rather rarely, especially in southern ostriches. Out of 20 Cape birds of mixed breeding only one showed an interruption, while in 20 mixed Nigerian birds a single break occurred in three cases and a double break in two. As the figures in



Text-fig. 2. Tarsi and feet of Northern Ostrich. The scutellation shows a strong break between the tarsus and the large, inner third toe and the beginning of a second break over the middle joint of the toe. The small, outer, fourth toe bears only a few scales and the claw shown is unusually well developed.

TABLE VIII.

Number of Scales in Tarsopodal Scutellation.

A. Continuous :		Right Tarsus and Toe		Left Tarsus and Toe	
1		53		55	
2		55		53	
3		57		56	
4		58		57	
B. With one break :					
		Tarsus	Toe	Tarsus	Toe
1		27	16	27	17
2		28	17	30	19
C. With two breaks :					
		Tarsus	Toe	Tarsus	Toe
1		29	5 8	29	5 8
2		30	5 9	31	6 9
3		32	6 9	31	7 11

Table VIII show, the breaks represent a definite loss of scales. Taken along with the other facts of degeneration in the foot, the losses are without doubt to be regarded as the first evidence of degeneration in the middle toe of the ostrich, the first, second and fifth having already disappeared and the small fourth being well on the way. The breaks evidently represent independent unit characters, retrogressive mutations, in course of introduction within the whole race, the process having gone a little further in the northern ostrich than in the southern.

In all ostriches the tarsal scutellation is now distinct from that over the small toe, only 8 to 10 scales occurring distally (Text-fig. 2). Comparison with other birds would, however, lead one to expect that the two series were originally continuous¹, as they are still in the great majority of ostriches with regard to the middle toe.

Breeding experiments prove that the breaks between the tarsus and middle toe are germinal in their nature. Where no break occurs in either of the parents the progeny also show no loss of scales. Thus in 13 cross-bred chicks from a southern cock and a northern hen, both with a continuous scutellation, no loss of scales occurred. When however one of the parents bears a break and not the other, then, as indicated below, approximately one-half of the chicks displays the loss, proving that the factor for the break is dominant but that the germ plasma is simplex or heterozygous with reference to it.

¹ At extensive series of birds' feet is shown on p. 425 of Sedgwick's, *Students' Text-book of Zoology*, Vol. II. 1905, where however the scutellation of *Struthio camelus* is erroneously represented, the scales of the small toe being depicted as continuous with those of the tarsus.

TABLE IX.

Scutellation in Parents and Chicks.

<i>Parents :</i>				No Break	Break
North African cock, No. 78				×	—
South African hen, No. 225				—	×
<i>Cross-bred Chicks :</i>					
No. 314	×	—
No. 315	×	—
No. 316	—	×
No. 318	—	×
No. 319	—	×
No. 320	—	×
No. 321	×	—
No. 322	—	×
No. 323	×	—
No. 300	×	—
No. 301	—	×

The heterozygous condition with regard to the break is what would be expected, assuming that the character is one which is in process of introduction within the race, and that it behaves in Mendelian fashion. At present the mutation is found in comparatively few individuals, and in a state of nature there is little chance that a bird showing the break would mate with another in a like condition, but rather with one having the scales continuous. If the change first took place in a homozygous duplex manner in a few individuals there is small likelihood that these would mate with others in like condition, but with nulliplex individuals. The first crosses would be dominant and simplex, and these mating with other nulliplex birds would give half simplex dominants and half nulliplex, which is what the experiments indicate. As shown below, certain significant results have been obtained on in-breeding some of the cross-bred birds.

TABLE X.

Scutellation in F_2 chicks compared with Parents and Grandparents.

<i>Parents :</i>				No Break	Break
North African cock, No. 9				×	—
South African hen, No. 225				—	×
<i>F_1 Crosses :</i>					
Cross-bred cock, No. 182				×	—
Cross-bred hen, No. 179				—	×
<i>F_2 Crosses :</i>					
No. 1	—	×
No. 2	—	×
No. 3	—	×
No. 4	×	—

In this case the original parents were a North African cock without any loss of scales and a South African hen with a single break. Of the four offspring reared three are without the break while it occurs in No. 179, the hen used in the experiment. From the mating of brother and sister four chicks were hatched, two of which had two breaks in the scutellation, one had only a single break and one had no break. The result may be regarded as highly suggestive that the inherent tendency towards the loss of scales can be accentuated by in-breeding, and degeneration thus accelerated along definite prescribed lines; for after the single break the next step in the loss is a second break over the middle of the toe. In the course of the investigations it has become evident that an inherent tendency exists in the ostrich towards the loss of various parts of the fore and hind limbs in a continuous determinate direction, as well as of its plumage, and it is not unlikely that by in-breeding the degenerative tendency can be accentuated. The accumulation of fuller data must however be awaited before the suggestion as regards the loss of scales can be regarded as more than tentative.

CLAW ON FOURTH TOE.

The claw on the small, fourth toe has for the most part disappeared from the ostrich, though it is occasionally present, more often on North African than on South African birds (Text-fig. 2). In 25 mixed northern birds it occurred on six specimens and was wanting on the others, while in 20 mixed southern birds it was found on only one individual. Everywhere it is feebly developed, especially by comparison with the big claw on the middle toe, and is always non-functional, never reaching the ground. Where best formed it projects for about half an inch from its socket, while at other times it is almost hidden in the integument, and can scarcely be felt with the finger; but all intermediate sizes can be obtained. Usually it is equally developed on both the right and left foot, though sometimes a difference is observed.

In crosses where both parents are without the claw the progeny are also devoid of it, though in a nest of 16 chicks from clawless parents a distinct claw appeared in one (Cross-bred No. 304). Where however one parent is clawed and not the other it appears in about half the progeny, showing that the clawed birds are dominant heterozygotes. Out of a total of 36 chicks hatched from breeding pairs, where one parent was clawed and not the other, the numbers were actually equal, 18 chicks clawed and 18 unclawed.

Thus, as in the case of the loss of scales over the big toe, the evidence is conclusive that the presence of the claw follows the Mendelian proportions for heterozygotes when breeding takes place. Where the parents are nulliplex as regards the factor no claw usually appears in the progeny, but where a clawed individual mates with a clawless the structure appears in practically half the progeny.

With the small proportion of clawed to clawless individuals among both northern and southern birds it is to be expected that most of the clawed ostriches will be heterozygous as regards the factor for the claw for in a mixed assemblage the chances of a clawed bird mating with another clawed one are very remote. A clawed bird will almost certainly be the progeny of one clawed and one clawless parent, and hence will be a simplex dominant, and when in turn mated with a non-clawed bird will give progeny half of which are again simplex clawed and half clawless.

DISCUSSION.

The northern and the southern ostrich illustrate in a clear manner how distinct species of animals may arise on the basis of germinal or factorial changes. It may be assumed that both are the descendants of an original stock in which the characters were all alike, and that in the course of time alterations have taken place in the germ plasm which give the marked differences now separating the two. Whether the changes have any adaptive value or not will be discussed later. Considered as a whole the broad genetical conditions are fairly simple, as no other representative of the Ratitae exists in Africa with which individual ostriches could have hybridised. As we know the two-toed ostrich to-day, it may be assumed to have evolved entirely within the continent, though in Tertiary times extending into Eurasia as far as Southern India, fossil remains having been found in the Siwalik deposits. That the two species had a common origin, and are not yet far apart, may be inferred from the fact that they interbreed and the offspring are fertile, also that similar degenerative changes are going on in the germ plasm of each (parallel mutations).

Factorial Constitution. The results from the crossing of the two species, though admittedly very incomplete, afford certain evidence as to the present germinal constitution of the ostrich, and indicate directions along which changes are taking place. Everything points to the distinctive characteristics of the two species as having separate factorial

representation in the germ plasma. The bald patch is a unit character which has appeared in the northern without any corresponding change in the southern bird. In all the individuals used for crossing it behaves in a homozygous manner, showing that it has become fully established throughout the northern race. It supports the presence and absence conception of genetic factors; the factor for baldness is present in duplex form in the germ cells of the northern ostrich but is absent from those of the southern. In cross-breds of the first generation it is simplex dominant, and in cross-breds of the second generation it is found to segregate.

The dimensions and colours of the ostrich, as well as the various features of the egg, have manifestly a more complex factorial representation than the bald patch. In the ancestral ostrich the factors for each of the characters were doubtless common to all the individuals, but germinal divergence has since taken place. The cross-breds serve to establish that each species now has its own separate factor or factors controlling its size, for those of the one are neither dominant nor recessive to those of the other. They do not constitute an alternative pair, a presence and an absence, as in the case of the bald patch. Hence in the zygote resulting from the cross-mating the simplex factors for both races are represented, the factor for the small size of the southern bird and that for the large size of the northern. In the resulting soma the two sets of forces are, as it were, each independently striving for expression, and naturally the individual cannot be both sizes at the same time, but appears as an intermediate, a resultant of the interaction of two distinct tendencies, one from the northern parent and one from the southern.

Similarly with the body colours. Each species may be deemed to have its own distinctive factors controlling its colours, for in crosses they do not behave as alternative pairs. In the cross-bred bird each factor endeavours to exert its pure specific influence, the result being a combination of the two, a colour which is intermediate and partakes of the nature of both; sometimes one factor may gain a slight ascendancy and sometimes another. The same conclusions as to factorial distinctness have been arrived at with regard to the various characters of the eggs which appear as intermediates in the F_1 crosses. The exact gametic constitution, as regards the dimensions, colours, and eggs, can however be determined only after a number of chicks of the second generation have been reared.

The factorial representation for the plumes must be complex to a

still further degree. As already indicated, the numerous "points" in the feather to which the farmer attaches importance behave in breeding practice as if each were under separate control. On the other hand we have abundant evidence that in ordinary breeding, and also in degeneration, each plume usually acts as an independent whole, as if some factor or group of factors controlled it in its entirety, irrespective of its numerous component factors. A complexity is however introduced by the presence of vestigial, imperfectly formed feathers, a few of which are sometimes found beyond the last fully developed plume in a row, and also as vestigial down on the wings and tail. Where vestiges occur during degeneration we can only surmise that the factors which control the whole plume may drop out piecemeal. They show that meristic structures do not always appear or disappear in their entirety. We may have a part of a feather as well as a whole.

Among the mixed assemblage of ostriches of the present day the genetic factors controlling the number of wing plumes in each bird may be regarded as heterozygous, for from any pairing we get a fluctuating series around some mode, less wide in some cases than in others; but if, as seems likely, it is found possible to extract pure lines for each of the numbers from 42 to 33 these will then breed true and may be expected to be homozygous.

The loss of scales from the middle toe evidently represents a germinal change which is actually in progress in both northern and southern birds at the present time. It proceeds along parallel lines in both races, its first manifestation being a loss of scales at the joint between the tarsus and toe and then another over the middle of the toe. The breaks represent a loss of structural parts of the foot, though they are dominant over continuity. As yet the germinal change involved in the first break has affected only a small proportion of birds and the second break a still smaller proportion. The other facts of degeneration in connection with the foot, the claw and scales over the small toe, and the loss of three toes, are taken to justify the assumption that the breaks represent still further degeneration which is in progress for the ostrich race as a whole. If, when first introduced, the change is a homozygous one, there is small likelihood that, in mating, the homozygote will meet with another homozygote. Until the mutation is introduced among a considerable number of birds the chances are that pairing will take place with a nulliplex individual, in which case all the progeny will be heterozygotes; these in turn are more likely to pair with a nulliplex, and as regards the break the offspring will be simplex dominant and nulliplex in equal numbers.

As already proved both the northern and the southern birds showing the mutation behave as heterozygotes in crosses.

Like the loss of the scales from the middle toe the loss of the claw on the fourth toe is a degenerative change in progress within the ostrich race as a whole; likewise it is germinal in its nature, and in crosses follows Mendelian lines. Also it can readily be admitted as part of an established degenerative scheme which has been going on in the foot for a vast period seeing that the first, second and fifth toes have already disappeared. The loss is however an individual one, not one affecting the race as a whole simultaneously; but compared with that of the scales the absence of the claw has reached a stage where by far the majority of the race are affected. The loss of the claw factor may be deemed to take place *de novo* in individual birds, and in course of time has affected larger and larger numbers. In crosses the presence of the claw is dominant over its absence, but, from the small proportion of individuals now possessing one, the chances of a clawed bird mating with another clawed bird must be very remote; hence, as the investigations prove, the few showing the claw are heterozygotes.

The fact that a germinal change, such as is involved in the loss of the claw or the scales on the toe, occurs in individual birds anywhere throughout the continent, strongly suggests that, whatever the degenerative influence may be, it acts on the germ plasm of the ostrich as a whole, wholly irrespective of environmental conditions. It gains expression *de novo* at different times in different individuals, but in the end affects all the members of the genus, as had happened in the case of the losses already sustained. The gradual loss of the scales, as well as that of the claws and feathers, indicate that retrogressive evolution is taking place piecemeal on strictly factorial lines, but in a continuous determinate manner as regards the race. If, as seems likely, we are to regard the baldness of the northern bird as the first step in the loss of the head plumage then we have an instance of the retrogressive changes affecting only one of the species (divergent mutations), while the loss of the scales and claw is in progress for the entire race (parallel mutations).

The parallelism of the changes going on in both species of ostrich is readily understood if we regard the two as having a common origin from the same germ plasm, with all its inherent tendencies. Many of the parallelisms of evolutionary changes found in other forms of life may also be deemed to be indications of a distant common origin for part at least of their germ plasm. Darwin in his *Origin*, p. 179, remarks: "As all the species of the same genus are supposed, on my theory, to have

descended from a common parent, it might be expected that they would occasionally vary in an analogous manner." All the parts of the germ plasm may be held to be the same for any "pure" species; some parts are changed and give us the distinctions between species; other parts differ in producing generic separations, and still more fundamental parts in giving ordinal, class and phylar distinctions; but corresponding changes may take place in the germ plasm which remains common to phylum, class, order or genus and so give rise to parallel mutations, the Analogous Evolution of Prof. H. F. Osborn¹, while divergent changes in the common germ plasm would produce Polyphyletic Evolution. In the ostrich specific changes have taken place, and others embracing the genus are in progress.

Adaptive Value of Changes. We may briefly consider whether the changes set up in the germ plasm, and expressing themselves in the soma, have any adaptive significance in the life of the ostrich. Following largely the teachings of Bateson and Morgan, few writers now are prepared to admit that germinal changes are a response to external environmental influences, or have appeared in response to some need of the organism, or have necessarily some selection value; rather it is held that they are dependent upon some intrinsic cause which may vary in different cases. Though we may not know much as to the conditions under which the changes are brought about till once effected and manifested in the body, we may discuss the question of their utility or otherwise to the individual and their influence on the evolution of the race.

The bald head patch on the northern ostrich is probably as neutral in its effect on the bird as it is possible for any character to be; it is impossible to think of it as exerting any beneficial or harmful influence compared with the feathered condition in the southern bird. Both races are equally successful. It should probably be regarded as the first step in the loss of the head covering, thus introducing plumage degeneration to this region of the bird, following upon losses which have already been effected over the legs, wings and body and which are presumably still in progress. In this case the baldness raises the whole question of the adaptive significance of the loss of plumage going on in the ostrich, only the bare facts of which can now be noticed. In chicks the outer surface of the leg, from the knee joint to the ankle, is well covered with feathers which practically all drop out before maturity is reached. The under

¹ H. F. Osborn, *The Age of Mammals*, Macmillan and Co., 1910, pp. 29—34.

surface of the wing is now practically naked. Only one row of under-coverts persists, and it is hardly ever complete, while rare survivals of members of the second and third rows indicate that the under surface was at one time well covered. The wing quills and upper-coverts are many less in number in some strains than in others, and the under covering of down for the body generally has all but disappeared, vestigial plumules appearing only around the base of the large feathers of the wing and tail. Probably no bird is at present so naked as the ostrich.

It is questionable however whether the loss of plumage hitherto has any serious influence on the well being of the ostrich. It is not incompatible with its present existence. But should the losses continue to a much further degree the absence of a protective covering may begin to be felt; while should the number of wing quills become still further lessened their inability to cover the usual quantity of eggs (12 to 16) during incubation may affect the number of chicks reared.

It is likewise impossible to ascribe any selection or utilitarian value to the colour differences between the northern and the southern ostrich. The light colour of the chicks, young birds and hens of the former compared with the dark colour of the latter can hardly be regarded as either advantageous or disadvantageous. The intense scarlet nuptial colouration of the northern cock as contrasted with the restricted scarlet of its southern rival has already been shown to give it no preference in the eyes of its sombre mate. On the natural veld it might possibly add to its conspicuousness, supposing any greater display value on the African plains could be thought of than that of an ordinary cock with his intense black body plumage, white wings, white neck and light legs¹.

In like manner the marked differences associated with the egg of the two races can scarcely be deemed to have any adaptive value. The bigger northern bird may be expected to produce a larger egg than its smaller southern relative, but from a selection point of view nothing in reason is to be said in favour of its rounder shape, smooth surface and absence of pitting, in comparison with the more oval shape and pitted surface of the other. Both are equally successful in artificial as well as natural incubation.

On mechanical grounds some advantage may accrue from the loss of the fourth toe, the claw of which has almost disappeared from the race.

¹ Ex-President Roosevelt in *African Game Animals*, has given much consideration to the question of protective colouration and considers (p. 181) that "Cock ostriches always show jet black, and are visible at a greater distance than any of the common game; the neutral tints of the hens making them far less conspicuous."

just as the loss of the first, second and fifth toes has for long conferred a mechanical advantage by transferring practically the whole of the leg movements directly to the big middle toe. If however the degenerative forces are so relentless as they appear to be and should next begin to attack the big toe there could then be no question of the ultimate influence of the genetic changes upon the well-being of the bird, for with the loss of all its toes it is inconceivable that the extinction of the ostrich would not be imminent. While the losses of the scales in the case of the single break on the middle toe and the still rarer double break are deemed to be the first steps in this direction, it is conceivable that they are for the time being advantageous in the flexions and extensions of the toe.

On the whole then no evidence is forthcoming that the differences between the northern and the southern ostrich have arisen because of any direct utilitarian consideration; and the same can be said of the retrogressive changes common to both. Having appeared, they may come to have an adaptive value; but even for this there is no support except perhaps as regards the loss of the toes. On the other hand there is much to indicate that, if the degenerative losses continue in the various directions already initiated, we may look forward in the dim future to the sad spectacle of a wingless, legless and featherless ostrich, if extinction does not supervene.

As already remarked it is impossible to resist the conviction that we have in the ostrich some intrinsic influence, slow but continuous in its action, which is bringing about the gradual loss in piecemeal fashion of the various parts of the wing and the legs as well as of the plumage, wholly irrespective of external influences or adaptive considerations. The losses are separate mutative changes so far as the individual is concerned, yet the result for the race is continuous, determinate degeneration along several directions. If, as seems to be the case, the losses hitherto have no adaptive value, then natural selection is in no ways concerned with them, though it will become operative when degeneration has proceeded so far as to interfere with the ordinary activities of the bird. Some adaptive value may be ascribed to the loss of the three toes from the foot, and also to that of the fourth which is in progress, yet it could hardly be conceded when, the same degenerative tendency continuing, the only remaining toe is attacked. It is manifest that an evolutionary change may be advantageous up to a certain point but disastrous when continued beyond.

The Nägelian idea that evolutionary changes have taken place as

a result of some internal vitalistic force, acting altogether independently of external influences, and proceeding along definite lines, irrespective of adaptive considerations, seems to be gaining ground at the present time among biologists¹. The degeneration phenomena presented by the ostrich appear to constitute as clear an example in support of it as could be adduced, while the genetical results seem to afford what has hitherto been lacking, namely, the direct application of mutation and Mendelian principles to continuous determinate changes, such as confront the comparative anatomist and the palaeontologist. The main evolutionary conception associated with mutation is fortuitous discontinuity, but in the ostrich we perceive how discontinuous changes in the individual may proceed along definite lines and result in determinate continuous evolution for the race as a whole. The loss of scales or single feathers in individual birds may seem to be nothing more than haphazard chance occurrences, but when considered for the race they indicate an orderly progress towards definite end results.

Establishment of Characters. If none of the changes which have taken place between the northern and southern ostrich have any selection value we may well enquire how the differences have actually become established. Undoubtedly geographical isolation as regards North and South Africa has played some part. Whatever intermediate forms may be found in the intervening areas, the ostriches in the more extreme parts of the continent must have evolved independently on one another for long ages, though not to such a degree as to bring about infertility between them. Some changes, such as the bald patch, and those connected with the size and colour of the body and the nature of the egg, are now distinctive between the two races, while others, such as the loss of plumage, the loss of the claw on the small toe, and of scales on the large toe, are common to the ostrich race as a whole.

Assuming the characteristics for the race to have been the same originally, and that the distinguishing features of to-day have no selection value, we may first enquire how, for example, such a unit character as the bald head patch has come to be dominant and duplex for the northern species, while altogether absent from the southern. On the factorial theory of variation we assume that some definite, hereditary change took place in the germ plasm of the northern ostrich, as a result of which the feathers fall out from the top of the head at a certain age. If we further admit that the number of ostriches for the area was con-

¹ C. B. Davenport, "The Form of Evolutionary Theory that modern Genetical Research seems to favour." *Amer. Nat.* Vol. L, Aug. 1916.

stant, that all were equally fertile and that breeding at random took place then a single pair of mature birds would on the average give rise to only a single mature pair. At the beginning we may allow that the mutation occurred in the germ plasm of, say, a single bird, and represented a double or duplex dose and was dominant. Such a bird mated with one in which the change had not taken place would give offspring all of which would be simplex dominants for baldness. Only two of the progeny would reach maturity and mating with two nulliplex individuals would give four mature birds of which two would have the factor simplex and two would be nulliplex. Thus on the conditions postulated the number of birds showing baldness would never increase beyond two and both would be simplex, that is, the new character would retain the same proportion throughout the history of the race. There would be no swamping of the character and no increase of individuals showing it. In the same manner if the factorial change took place in the germ plasm of a number, x , of birds simultaneously, their influence on its introduction would be to the extent of $2x$, if all the progeny were simplex. If matings took place between simplex pairs instead of between simplex and nulliplex then the result at maturity would be one duplex dominant, two simplex dominants and one nulliplex on the average for each two pairs; in other words, the mating would result for the time being in a loss of one-fourth of the number bearing the character, but the original number would be restored if the duplex dominant paired with a nulliplex, for two simplex individuals would result.

Thus under the conditions stipulated—a new character of no selection value, a stable population, free intermingling and equal fertility—conditions which it must be admitted are closely approximated in the natural life of the ostrich, the complete introduction of a new unit character in duplex form would occur only by the germinal change taking place as many times as there are individuals making up the race. On the other hand the character could be introduced in a simplex form by the change being effected in half the number of individuals. It follows that unless a new character has some selection value it cannot be bred into a race; it must be introduced *de novo* for each homozygous increase and half the number of times for the heterozygous increases.

As regards the bald patch therefore the germinal change must have been effected as many times as there are individuals making up the northern race, for the experiments have proved they are all homozygotes. We can scarcely conceive that the alteration would be carried out simul-

taneously in all the individuals, presumably it affected a few at first and others gradually. For a long time in the history of the race some of the birds would be duplex for baldness, some would be simplex and the rest would be nulliplex. Mr G. H. Hardy (Punnett's *Mendelism*, 1911, p. 136) has shown that under conditions such as are stipulated for the ostrich the population would rapidly fall into a stable condition with regard to the proportion of the three forms, whatever may be the proportion to start with. If the population consists of p homozygotes of one kind, r homozygotes of the other kind and $2q$ heterozygotes then he points out that such a population would be in equilibrium for a particular factor so long as the condition $q^2 = pr$ is fulfilled. The proportions which satisfy the equation are exceedingly numerous and in case of any disturbance of the equilibrium, as by the appearance *de novo* of the character, it will be restored after a single generation.

From the foregoing we gather how little effect under natural conditions the importation of the hundred and thirty-two northern birds would have upon the southern race if the population of the latter were stationary. If all the northern birds reached breeding age and mated with the southern they would in the end give rise to only double the number of simplex, bald-headed ostriches, and the number would neither increase nor decrease. How far the other characters—dimensions, colour and nature of the egg—would influence the southern race cannot be determined until their actual factorial values have been worked out.

The conditions represented by the loss of scales from the middle toe and the claw on the fourth toe further help in an understanding of how mutative changes are introduced into a large assemblage. It is seen that the loss of scales has occurred in individuals of both the northern and southern birds, though more frequently in the former. The change however must have appeared independently north and south, for the distinctness of the other characters, especially that of baldness, proves that no intermingling of the two species has taken place towards the extremities of the continent. Appearing at first in comparatively few individuals, and presumably in duplex dominant form, it is most unlikely that a bird having the break would mate with another showing the same loss, but rather with one in which the scutellation was continuous. The duplex bird mating with a nulliplex would give F_1 progeny in all of which the break would appear, while germinally they would be simplex. The simplex condition would tend to be retained until such time as the character became prevalent and opportunity occurred for a simplex to meet with a simplex, when a duplex condition would arise. It is significant

however that in all cases where an ostrich showing the break has been paired with one in which it is wanting, the bird has proved itself to be heterozygous, giving progeny of which approximately half display the break and half the continuous scutellation. Thus while baldness is a mutation fully established for the northern race and germinally duplex, the loss of scales from the third toe is a mutation only partly established and germinally simplex. A character in course of introduction within a race will for a long time be mainly in a simplex or heterozygous form; later, as the mutation appears *de novo* in more and more individuals, the population will tend to consist of duplex, simplex and nulliplex birds, until in the end all will be duplex or homozygous.

As regards the race as a whole the claw on the fourth toe reveals conditions somewhat similar to those of the loss of scales over the middle toe, but is a character which has almost disappeared. Experiments have shown that the presence of the claw is dominant over its absence, and matings with a nulliplex individual give progeny half simplex dominant and half nulliplex, proving that the clawed individuals are heterozygotes. This again is what would be expected considering the small proportion of clawed birds, and the remote likelihood that a heterozygote would mate with a heterozygote. If only heterozygous individuals are to be found then any further loss of the factor will presumably take place as a simplex, and to be completely lost to the race the change must take place as many times as there are heterozygous birds. As for the introduction of a new character so for the loss of an existing character, it cannot be bred out under the conditions postulated, but must drop out germinally.

Specific distinctness of Northern and Southern Ostrich. Whether the northern and the southern ostrich are to be regarded as separate species, or only as sub-species or varieties of a single species, raises the ever-recurring, but undefinable question as to what constitutes a species. In the foregoing we have available all the data which the systematist could possibly desire to enable him to reach a decision. A germinal character, baldness, occurs in one, but is wanting in the other, while the dimensions and colours of the body as well as certain features of the egg are also distinctive and germinal. The characters are retained when the members of one race are subjected to the same environmental conditions as the other, showing they are not dependent upon external circumstances. They can all be regarded as distinct elementary characters in

the De Vriesian sense, and the combinations might well be held to warrant us in regarding the two as specifically distinct.

On the other hand the birds are proved to interbreed freely, and the offspring are fertile, both *inter se* and with the parental forms. The fact that similar degenerative processes—loss of plumage, scales and claws—are proceeding in both also points to a close germinal relationship. In the opinion of many systematists the physiological fact of fertility alone would be deemed to justify specific unity.

The ostrich ranges over all the habitable parts of Africa and there is every likelihood that in intermediate areas between north and south a mingling of the two races goes on, producing a mixed population, composed of all possible combinations of the two sets of characters. Thus in the East African Ostrich, *S. massaicus*, as the writer has found in visiting the ostrich farms in British East Africa, the colour of the hen and immature cock is a cream yellow while the mature cock has the head, neck and legs scarlet, and the birds are somewhat larger than the southern. The bald patch is present and the eggs are pitted. The Somali Ostrich, *S. molybdophanes*, is described as a smaller, darker bird than the southern, but the bald patch is wanting, and the colouration is like that of the southern and the eggs are pitted.

If the entire ostrich population of Africa were gathered together we are probably justified in thinking that all intermediate forms would be forthcoming between typical northern and southern birds. An exception would occur however in the case of the bald patch, for however much inter-crossing had taken place the character would never be intermediate, but would be wholly present or absent; and though the dimensions, colours and egg characters appeared in varying intermediate degrees as a result of crossing we should still have the knowledge that their distinctive nature could be extracted by selective breeding. When discussing intermediates the possibility of segregation should always be borne in mind. In the present instance, where all the facts are known, intermediate forms grading from one species to the other have no direct bearing on the question of specific distinctness. Among the African fauna especially, experimental breeding would probably establish that many so-called species and sub-species, often founded upon one or a few specimens, are in reality intermediates or hybrids of other species.

So long as we have the facts before us it is of small moment whether we regard the northern and southern ostriches as distinct species or not. It becomes a matter of individual predilection whether greater importance should be given to somatic differences or to physiological

similarity. Without being biassed in either direction it appears to the writer to make for convenience to regard them as distinct under the names bestowed by Linnaeus and Gurney.

DESCRIPTION OF PLATE VII.

- Fig. 1. Outer surface of fore-wing of ostrich, with plumes clipped off, to show arrangement of wing quills on upper (post-axial) border and rows of upper-coverts. The claw on the bastard wing is not visible.
- Fig. 2. Under surface of fore-wing of ostrich which is naked except for the single, incomplete row of under-coverts.
- Fig. 3. Egg of North African ostrich (to the left) and South African ostrich (to the right).



DOUBLE FLOWERS AND SEX-LINKAGE IN *BEGONIA*.

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(With Plate VIII.)

BEGONIAS are monoecious plants, having their flowers arranged in axillary cymes. In normal plants the flower which terminates each dichasium is a male; and, in the simplest arrangement, upon either side of this stands a female. For one or both of these females may be substituted a continuation of the inflorescence, which again at each dichasium ends in a male, this system being indefinitely repeated.

Since doubleness affects only those flowers which stand terminally, being that is to say in normal plants *males*, an investigation of the inheritance of this condition offered attractions, as being not unlikely to throw light on the genetics of sex. In passing it may be remarked that since a female flower can be replaced by an inflorescence, whereas a male flower is not thus replaceable, from these morphological relationships we are led to infer that the female flower contains something that the male has lost. The male flower may be thus compared to a recessive, dropped out of the inflorescence which can be produced further in the heterozygous state.

The investigation was begun in 1908 by fertilising the normal female flowers of double Begonias with pollen from singles of unknown origin. Subsequently further crosses were made between doubles and a horticultural strain of singles which was declared to have bred true for some generations. The results have been full of complications such that, after many years work, it has become evident that no simple factorial scheme is followed, and that segregation in regard to single and double flowers must in these plants be a process liable to considerable irregularity. In general the single is a dominant as Bond also found. The recessive doubleness reappears in F_2 , but the numerical proportion of F_2 doubles is low and fluctuates widely. There are many transitional forms, which render accurate classification and enumeration impossible.

and not very rarely several of them may appear on the same plant. Some of the more interesting of these forms will be spoken of later.

An average of many F_2 families gives about 1 double in 32, but in several large families no doubles at all appeared, and this average has certainly no general significance. From F_1 plants crossed back reciprocally with various doubles, similar irregular numbers were obtained and no approximation to analysis could be made. To render the composition of these families intelligible lengthy descriptions would be required and little purpose would be served by the publication of such details. Their interest lies chiefly in their value as an indication that in regard to a character which in so many plants is distributed genetically according to strict allelomorphic rules, great irregularity may elsewhere prevail. Whether this irregularity is in any way connected with the monoecious structure of *Begonias* cannot of course be declared. Such a conclusion is by no means improbable.

The purpose of the present paper is to make known a curious discovery which resulted when *Begonia Davisii* was brought into the series of experiments. The plant is one originally found in Peru by Mr Davis, collector for Messrs Veitch, and first flowered by them in the year 1876¹. Inasmuch as this is a real species, breeding perfectly true on self-fertilisation, it seemed suitable for use as a reliable single for crossing with doubles. When however these crosses were made it was found that any double fertilised by pollen of *B. Davisii* gives *only double-flowered offspring*—405 plants have been thus raised, and of these only 18 are recorded as having less than complete doubling. The male side of *Davisii* is therefore exclusively double-bearing. Since the same plant fertilised with its own pollen gives only singles, the female side must be inferred to be exclusively single. Tested however with the pollen of a double, it gave a result which we cannot satisfactorily interpret. Fertilisation with pollen of doubles cannot always be accomplished, since thoroughly petalodic flowers do not produce pollen. A good many doubles nevertheless when starved or poorly grown do produce anthers and pollen, as for example the well-known double called in horticulture *Begonia Lloydii*. *B. Davisii* ♀ fertilised by *Lloydii* ♂ gave 72 thorough singles and 42 with traces of petalody, a condition we have not yet seen in *Davisii* itself. The genetic nature of these slightly petalodic plants is not clear. If they can be formed when the pollen of *Lloydii* is used, we should expect them to appear when *Davisii* is fertilised with its own pollen, for this pollen used on

¹ See Wynne, *The Tuberous Begonia*, 1888, p. 16 and *Bot. Mag.*, t. 6252.

doubles gives scarcely anything but extreme doubles. Slightly petalodic plants came also occasionally, among large numbers of singles, in families raised from *Darvisii* female fertilised with pollen of heterozygous plants (F_1 from double \times single σ). When the pollen of *Darvisii* is used on such F_1 plants the proportion¹ of recessive, double-bearing, ova, of course appears; and since perfectly reliable pollen of doubles is difficult to obtain, the pollen of *Darvisii* may be substituted for it.

After discovering the peculiar genetic constitution of *Darvisii* we naturally expected that the results of reciprocal crosses made between doubles and F_1 plants (from double \times single) would at least sometimes show linkage of doubleness with either the male or the female side. For this investigation a considerable amount of material is now available and we are satisfied that in general heterozygotes do not show any regular phenomenon of this kind. In contrast however to the usual absence of consistent sex-linkage, one plant raised from the female side of *Darvisii* fertilised by *Lloydii* was proved to possess such sex-linkage, though less complete than that of *Darvisii* itself. The plant, self-fertilised, set badly and only 2 plants (singles) were thus raised. As regards its female side we have the evidence that with *Darvisii* pollen it gave 11 singles, and with *Lloydii* pollen 5 singles and 1 slightly petalodic, from which it may be inferred that the ovules were at all events predominantly single-bearing. The male side tested on *Lloydii* gave 27 doubles, 14 half doubles and 5 slightly petalodic (see Nos. 23—26 in Table on p. 206).

As to the presence of sex-linkage in other heterozygous individuals the evidence is as yet conflicting. Some plants show it, whereas others do not, and we cannot as yet perceive any circumstance either in the way in which the plants were made up or in any other respect which accounts for these differences. We give in the Table (Nos. 31 to 45 on p. 207) specimens of these various behaviours.

The case naturally recalls other examples in which sex-linkage has been observed in plants. In three of these the male side has been specially distinguished as being associated with the *recessives*, though whether this is an accidental circumstance due to the way in which the plants were originally bred cannot yet be declared, but in *Petunia*, as shown by Miss Saunders singleness, the dominant, was carried by all the pollen-grains, and by some only of the ovules of the single-flowered plants. In *Matthiola* the pollen was all double and for the most part carrying cream plastid-colour (Saunders); and in a plant of *Campanula carpatica*

¹ This proportion, as Table II exemplifies, is apparently quite irregular.

and its descendants, the pollen bears white flower colour and femaleness, the factors for blue and for the hermaphrodite condition being carried by the ovules (Pellew). In this case as in *Begonia* the sex-linkage was not general but special to a particular plant and its descendants. Of these examples the plastid-colour is the only one in which the converse combination has yet been built up, though perhaps the others may hereafter be obtained¹.

The condition in *Oenothera* "*velutina*" described by de Vries must be very similar, the recessive dwarf character being carried by the pollen. In the corresponding case of *Oenothera* "*laeta*" the evidence also points to the pollen being all dwarf, and to the existence of a mixture of tall and dwarfs among the ovules, in spite of which the plants do not throw dwarfs on self-fertilisation. This absence of dwarfs on selfing constitutes a puzzle exactly like that of the presence of slightly petalodics in *Davisii* \times double σ^2 .

When in hermaphrodite flowers the male and female sides are genetically distinct we feel fairly sure that the segregation of these allelomorphs occurs not later than the formation of the anther-rudiments, but in *B. Davisii* it presumably happens even earlier and not later than the formation of the male flowers. Those who incline to regard the reduction division as the stage at which alone segregation can be effected may no doubt be tempted to suggest that in *B. Davisii*, for instance, pollen grains bearing the dominant factor are in reality formed but in some unexplained way fail to take part in fertilisation. As a mere suggestion of a possibility that theory cannot as yet be absolutely excluded, but in this special example it is more than usually difficult to accept, since the pollen of *B. Davisii* is to the eye exceedingly uniform and regular. There are none of the shrivelled grains which are generally looked upon as the bearers of missing elements. Though less significant, the absence of seeds partially defective is also noticeable.

In applying the term sex-linkage to such cases as this I am following

¹ Since in the original form the ovules were mixed and the pollen was all recessive, the "converse" might appear in one of two forms. Either (1) the ovules might be all dominant and the pollen mixed; or (2) the ovules might be mixed and the pollen all dominant. As Miss Saunders's plants were tested by self-fertilisation and not by crosses with recessives it cannot yet be declared which of the above possible constitutions they possessed, but she considers there is a presumption that they were really arranged on the second of the two plans. (See Saunders, *Jour. Gen.* iv. pp. 332 and 359 and compare Pellew, *Jour. Gen.* vi. p. 320, &c.)

² For a discussion of these *Oenothera* cases see W. Bateson, *Problems of Genetics*, 1913, p. 113.

the suggestion made by Miss Pellew in her discussion of "Types of Segregation". The propriety of the comparison between the association of a character with one of the sexes in the case of a hermaphrodite plant and the phenomenon in bisexual animals commonly called sex-linkage may be questioned, but until we know more precisely how sex in animals is related to the phenomena in the flowering plant, no unjustifiable assumption is made and no serious confusion can be caused by their use. If, following one method of interpretation, we regard pollen-mother cells, being the latest diploid stage, as the equivalent of male animals, we can reasonably speak of the character—here doubleness—carried by the pollen-grains, as linked with maleness, and singleness as linked with femaleness. The comparison, though not certainly valid is at present defensible. The relation of the hermaphrodite to the dioecious condition, whether in animals or in plants has not yet been represented by any factorial scheme which is thoroughly satisfactory. On a survey of the various sexual arrangements followed among plants we meet a difficulty in attempting to choose any fixed moment common to all the cycles, which can serve as a starting point for the institution of homologues. The difficulty is intensified when we proceed to the case of animals. One obvious suggestion is that the reduction-division provides such a common fixed point. Though I am not disposed to look upon that event as the only occasion on which Mendelian segregation is effected, I readily agree that many segregations presumably do happen then, especially that by which sex is usually determined among animals. Such observations however as those of the Marchals and the new evidence discovered by Collins² show almost beyond question that even within the group of Mosses sex-segregation may occur at different moments in the different cycles.

With equal propriety we may regard the actual gametes as the fixed point common to all and therefore homologous in all the cycles, but we have still to face the difficulty that such a critical segregation as that which determines sex (and probably others) may be sometimes effected at the reduction-division, sometimes before it, as at least in monoecious flowering plants, and sometimes after it as in Collins's *Funaria*.

The facts practically drive us to the conception that the ordinal position of the reduction-division can be shifted in the cycle, and that segregations which in some cycles precede reduction are in other cycles

¹ *Jour. Gen.* 1917, vi. p. 319.

² In the present number of *Jour. Gen.*

postponed until reduction has been already undergone. The problem is not unlike that so often raised by the differentiation of parts composing a meristic series. In one Lizard the n th vertebra carries the pelvis and undergoes special modification. In another Lizard the vertebra thus differentiated is the $n + m$ th in ordinal series. Morphologists have long discussed whether in allotting homologies among vertebrae we should be guided by the differentiations, or by the ordinal positions. When once the true nature of segregation and differentiation is understood the question is seen to lose all significance¹, and having no precise meaning is incapable of being answered. For the individuality of the segments is not respected or maintained in variation, nor are differentiation and numerical change necessarily interdependent. We may easily satisfy ourselves that the numbers may vary and that within considerable though unascertained limits the functions and differentiations of the segments may be redistributed. I can scarcely doubt that we must similarly interpret the series of divisions and differentiations of which the life-cycles consist.

In the Tables we represent the plants as of five classes. *Singles* are those in which the male flowers have not been seen to have more than the four normal petals. The *slightly petalodic* class have generally one or two, though occasionally rather more extra petals or petalodic anthers. These two classes cannot be quite strictly instituted, and plants having flowers of both kinds are common. The *half-double* class ranges from the slightly petalodic to the really double, but nevertheless it is a fairly uniform class. *Doubles* and *full doubles* are not essentially distinct, but the term *full* was applied only to flowers in which the petals were very numerous and close.

As was stated above, peculiar and transitional forms are common. In particular some difficulty is caused by structures consisting of female and male flowers imperfectly resolved from each other². Such flowers can generally be recognized by examination of the bracts, but when this condition of imperfect resolution is combined with some degree of petalody the degree of doubling cannot be determined with much confidence.

Since double flowers stand terminally, that is to say in the male position, we supposed at the beginning of these experiments that double flowers were necessarily petalodic males. Happening however to examine

¹ See *Problems of Genetics*, p. 66.

² Noticed by Bond, *Jour. Gen.* iv. 1915, p. 341.

the variety called Graf Zeppelin, we were struck by the fact that the double flowers, though terminal, are in reality modified *females*. There is no inferior ovary, but at the bases of the petals are masses of exposed ovules¹. This arrangement is normal for the variety and gives it a most characteristic appearance. Further search among double Begonias showed that many are in essentially the same condition, though the amount of ovules developed varies greatly. Probably most of the fine exhibition blooms are modified female flowers, though in them the ovular tissue may be reduced to a mere trace at the base of occasional petals.

Whether any of these plants are altogether incapable of producing anthers, however much they may be starved, we do not know. Our experience inclines us to think that some plants cannot produce anthers, though we have certainly seen thoroughly double flowers of the ovule-containing kind on plants which had borne double males containing anthers. But apart from this question we can easily recognize a class of doubles, of which *Lloydii* is a good instance, in which the double flower is essentially male; and though they may be fairly perfect doubles when well grown, this kind of double can readily be starved into producing pollen. The view that plants, *e.g.* Graf Zeppelin, in which the terminal flowers are female, instead of male as normally, may be *homozygous* females is rather attractive, but we see no means of testing it; nor if such an idea could be entertained, would it at all account for the fact that in a full double which must certainly be accepted as a recessive, homozygous in doubleness, the normal female flowers standing in the lateral positions are single. Beyond this point we see as yet no means of pursuing the analysis.

Since *B. Davisii* is a genuine wild species and bears exclusively single flowers, the conclusion to which our observations have led us, namely that its male side is genetically all double, seems not a little remarkable.

¹ Flowers having this structure were referred to by Wynne, *l.c.*, p. 13, and parts of them are figured by Bond, *Jour. Gen.* iv. Pl. XVI. Their morphology is obscure, but it seems natural to regard the carpellary walls as represented by a mass of petals. We have never seen a normal female standing in the male position.

DETAILS OF EXPERIMENTS RELATING TO *BEGONIA DAVISII*.

Reg. No.							
1	—	<i>Davisii</i> selfed, 4 families, 200—300 raised all true to type					
2	—	<i>Lloydii</i> selfed, 45 true					
<i>Various doubles fertilised by Davisii ♂.</i>							
			Single	Slightly petalodic	Half double	Double	Fully Double
3	—	<i>Lloydii</i> × <i>Davisii</i> ♂ ...	—	—	—	—	32
4	49/13	Graf Zeppelin × do. ...	—	—	—	—	28
5	20/14	49/13 (as above) double × do.	—	—	6	3	16
6	30/15	20 ⁹ /14 (as above) double × do.	—	—	3	—	14
7	28/13	A double × do. ...	—	—	—	—	139
8	4/14	28/13 (as above) double × do.	—	—	9	8	78
9	30/18	A double × do. ...	—	—	—	—	13
10	22/14	Argus × do. ...	—	—	—	—	17
11	25/14	Hollyhock × do. ...	—	—	—	—	8
12	27/15	Louis Boucher × do. ...	—	—	—	—	7
13	85/18	Fleur de Chrysanthème × do.	—	—	—	4	—

Davisii ♀ fertilised by double.

14	10/17	<i>Davisii</i> ♀ × <i>Lloydii</i> ♂ ...	72	42	—	—	—
----	-------	---	----	----	---	---	---

Reciprocal crosses with a half double.

15	4/17	<i>Davisii</i> × 37/14 half double ...	39	15	—	—	—
16	5/17	37/14 half double × <i>Davisii</i> ...	—	3	2	17	109
17	15/15	The same half double 37/14 selfed	1?	28	49	—	8

Reciprocal crosses with a heterozygous single.

18	41/16	<i>Davisii</i> ♀ × 2 ²¹ /14 hetero- zygous single	105	1	—	—	—
19	3/17	2 ²¹ /14 heterozygous single × <i>Davisii</i> ♂	68	14	5	17	23
20	42/16	The same heterozygous single 2 ²¹ /14 selfed	42	4	—	—	—
21	2/17	2 ²⁰ /14 heterozygous single × <i>Davisii</i> ♂	67	9	4	34	24
22	36/16	The same 2 ²⁰ /14 selfed ...	45	10	4	1	1

The following are tests of two plants bred in Experiment No. 14, *Davisii* ♀ × *Lloydii* ♂. In the first group 10²/17, a *single*, was used: in the second group 10⁵/17, a *slightly petalodic*, was used. In both, the male side proved to be predominantly double.

Reg. No.			Single	Slightly petalodic	Half double	Double	Fully double
23	12/18	10 ² /17 × <i>Davisii</i> ♂ ...	11	—	—	—	—
24	13/18	<i>Davisii</i> ♀ × 10 ² /17 ...	6	—	—	—	—
25	14/18	10 ² /17 × <i>Lloydii</i> ...	5	1	—	—	—
26	15/18	<i>Lloydii</i> × 10 ² /17 ...	—	5	14	14	13
27	20/18	10 ⁵ /17 × <i>Davisii</i> ♂ ...	14	8	—	—	—
28	22/18	Do. × <i>Lloydii</i> ♂ ...	2	2	1	—	—
29	23/18	<i>Lloydii</i> × 10 ⁵ /17 ...	1	1	5	—	11
30	24/18	Graf Zeppelin (fully double) × 10 ⁵ /17	—	—	1	—	—



The flowers and leaves of *Begonia Davisii*.

Experiments illustrating behaviour of various heterozygous singles (33¹/17, 17¹/17, 16²/17, 34¹/17).

In 33¹/17 the single factor went in from the mother's side, and there is a clear indication that the pollen was predominantly double; but in 17¹/17 and 16²/17, similarly bred, the pollen was predominantly single. In 34¹/17, the exact reciprocal of 33¹/17, the single factor went in from the father's side, and the numbers though insufficient, do not suggest sex-linkage.

Reg. No.			Single	Slightly petalodic	Half double	Double	Fully double
31	71/18	33 ¹ /17 selfed	45	6	—	—	—
32	72/18	Do. × 7 ¹ /17 double ♂ ...	43	7	6	2	—
33	73/18	35 ⁹ /17 double × 33 ¹ /17 ♂ ...	31	25	27	2	43
<hr/>							
34	53/18	17 ¹ /17 × <i>Lloydii</i> ♂	18	—	—	—	—
35	54/18	<i>Lloydii</i> ♀ × 17 ¹ /17	4	2	—	—	—
36	56/18	35 ⁹ /17 double × do.	4	—	1	1	3
37	57/18	35 ⁷ /17 double × do.	41	8	2	2	7
<hr/>							
38	34/18	16 ² selfed	27	—	—	—	—
39	35/18	Do. × <i>Lloydii</i>	55	17	—	11	17
40	36/18	<i>Lloydii</i> × 16 ² /17	88	24	—	3	3
41	37/18	Graf Zeppelin × do.	56	7	1	—	4
42	38/18	35 ¹⁰ /17 double × do.	55	23	15	—	14
<hr/>							
43	74/18	34 ¹ /17 selfed	73	4	—	—	1
44	76/18	Do. × 35 ³ /17 double	47	16	11	—	16
45	75/18	35 ⁸ /17 double × 34 ¹ /17 ...	6	2	3	—	1

Plate VIII shows the flowers and leaves of *Begonia Davisii*.

THE INHERITANCE OF WING COLOUR IN LEPIDOPTERA.

I. *ABRAXAS GROSSULARIATA* VAR. *LUTEA* (COCKERELL).

By H. ONSLOW.

(With Plates IX and X, Table and twenty-five Text-figures.)

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I. INTRODUCTION.

AMONG the Heterocera varieties which exhibit changes both of colour intensity and colour quality are not uncommon. The former may be broadly classed as melanic varieties. The latter, which are less

frequent, often show a change from red to yellow, or from yellow to white, the ground colour alone being affected. I am at present investigating several examples of both these varieties.

The present communication deals with the yellow variety of *Abraxas grossulariata*, in which the markings are normal, and bright orange or yellow replaces the usual white ground. Little or nothing is known of the pigments involved in such changes, or indeed of any insect pigments, with the single exception of the white and yellow colours of the Pieridae, which Hopkins¹ has shown to be due respectively to uric acid, and to some unknown reduction product of the same acid. It is possible that a similar relationship exists between the yellow and white pigments of *A. grossulariata*; and if this be so, an enzyme may be found to control the changes in both cases. I hope to be able to undertake a chemical study of the pigments at an early date.

The experiments were undertaken in order to investigate the genetic relationship of the white and yellow ground colours in crosses between *A. grossulariata* and its variety *lutea*. The type insect is usually papery white (see Plate IX, Nos. 25 and 26), or sometimes a very pale shade of cream; and the depth of the yellow ground in *lutea* varies considerably. Preliminary crosses showed that the white colour of the type insect was only completely dominant if the yellow of the variety was pale; and it became evident very soon after the experiments were commenced, that this white colour did not behave as a simple Mendelian character. It is in fact an example of a character which varies more or less continuously; and such cases frequently present problems for which it is difficult to find a satisfactory explanation. As a rule, the F_1 heterozygote from *lutea* \times *grossulariata* is not white, but can be distinguished from the type by a tinge of yellow which occasionally reaches an appreciable depth. The total range of variation in all crosses is considerable, and extends from the papery white of the type insect, through the palest shades of lemon, to a bright reddish orange (see Plate IX, Nos. 1—26).

Since the material could not at once be divided into discontinuous classes, several attempts were made to grade the insects and to place them by inspection in four arbitrary groups. This method had to be abandoned as no reliability whatever could be placed in the judgments, even when these were made under similar conditions of lighting, etc. An instrument of some kind for determining the colour appeared essential, and several were examined for this purpose. The colour-

¹ F. G. Hopkins, *Phil. Trans.* Vol. 186, Part II. p. 661, 1895.

wheel, notwithstanding its mechanical disadvantages, has much in its favour, but was finally abandoned for a commercial instrument called the "Tintometer." The reason for this selection was that with the colour-wheel there is no method of defining or recording the colours of the discs employed: it is therefore impossible for any future observer to reproduce these discs, and consequently the colours of any readings taken with them. The "Tintometer" however supplies a unit, which though arbitrary, is recoverable and satisfies the other essentials of a standard. Also, it is placed on the market at a moderate price. The scale consists of a series of coloured glasses, carefully dyed and standardised by comparison with the glasses of other scales, so that several opportunities for error are introduced. For this reason it must be admitted that the colour-wheel would be preferable, since with it there is but one judgment to introduce error. Unfortunately, no series of standard colour discs can be procured. They should be based on some physical constant such as wave length, so that they could be checked easily in case of fading.

It may be added that sometimes the colour-wheel can be used with advantage combined with some simple optical arrangement such as is provided by the "Tintometer," to secure conditions of equal illumination.

II. THE "TINTOMETER"¹

The "Tintometer" (Fig. 1) consists essentially of a rectangular tube *B*, slightly tapered and about 10 inches long. At the narrow end there is an eye-piece *A*: at the other end there are two apertures which admit light. The tube is mounted on a base to which it is inclined at an angle of about 45°. Just above the apertures there are two rows of grooved slots *G*, which receive the graded standard slips of coloured glass *F*, for intercepting the beams of light before they reach the eye.

The apparatus is used as follows: a piece of mirror is put immediately under the two apertures and the instrument placed in diffused daylight, preferably from a north window. The instrument is now moved until both fields of view are equally illuminated: all objects such as window-sashes, trees, etc., being avoided. The unknown coloured object is then placed under one aperture, and the specially prepared white background *C*, made of firmly compressed plaster of Paris, under

¹ Further details for the use of this instrument for other purposes are to be found in *Measurement of Light and Colour Sensations* (George Gill and Sons) and *Light and Colour Theories* (E. & F. N. Spon, Ltd.) by J. W. Lovibond, the inventor.

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the other. The colour slips *F* are then placed in the grooves until an equivalence of colour has been obtained in both fields of view. The coloured object may of course be placed indifferently on either side without affecting the result. In making the measurements here recorded a low power lens *D*, magnifying about three diameters, was

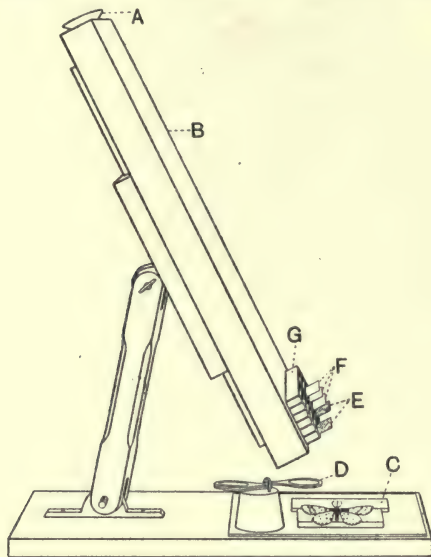


Fig. 1. The "Tintometer" arranged for reading the colour of an insect.

A. Eye-piece. B. Tube. C. Compressed plaster of Paris background. D. Lens. E. Cardboard diaphragms. F. Standard coloured glasses. G. Slots to hold glasses.

placed between the insect and one aperture. A variety of different sized diaphragms were also cut in black card. These were placed in the metal slots *E* in order to mask the black markings of the insect, which might otherwise have interfered with the colour determinations by introducing a contrast effect. A second diaphragm of the same size was placed on the opposite side from the insect in order to equalise the two beams of light.

The colour scale.

The colours of the glass slips used are yellow, red and blue. The scale consists of twenty units, each divided into ten parts, every one of these parts being again divided into a further ten fractions. It is however only with the paler colours that an increment of 0.01–0.05 becomes appreciable. These three colours can be combined to form any other colour required. Thus, one unit of red + one unit of yellow = one

unit of orange; and a yellow-orange colour may be made by combining one unit of red with two or more units of yellow. Further, one unit of red + one unit of yellow + one unit of blue = one unit of neutral tint, or black.

It is affirmed by the makers that the three colour-units which go to make up the neutral tint are equal, that is to say, when three different coloured units are combined there is no residual colour. All units and fractions are said to be checked by this test, and, further, all the glasses of one set are interchanged with those of another and the units verified by cross checking. To enable observers to verify their scales the makers publish colour readings, obtained by dissolving a known weight of a pure substance such as potassium-ferricyanide in a given volume of water, a known depth of which can be examined in cells of different thicknesses. The only objection to this procedure is that even solutions of the most stable substances such as picric acid are comparatively inconstant, and may show either a fading, or an increase of colour on standing.

In order to obtain the colour measurement of a given insect it should be pinned upon a strip of cork and so placed that the desired portion of the wing, magnified by the lens, comes immediately under one aperture. A suitable diaphragm is then chosen to cut out most of the black markings, while leaving exposed the central yellow portion of one wing. A red glass slip of a certain value is next chosen, and this is combined with a yellow slip, representing about twice as many units. Such a combination will give a bright orange which may be either too intense or too dilute. By the selection of suitable units or fractions both the red and the yellow are alternately increased and decreased, till a colour exactly matching the wing is obtained. Care should be taken to limit the length of each observation to five seconds, in order to avoid the disturbing effects due to fatigue. It is often found impossible to obtain a perfect match with the red and yellow colours only, owing to the fact that the colour of the wing is desaturated by the addition of black. In this event the exact colour equivalence may be obtained by adding some fraction of a blue unit, e.g. when the units on the slips of each colour employed in obtaining a perfect match are added together, the total may be found to come to:

Red	Yellow	Blue
5.6	: 9.8	: 0.4

These colours will not however be the same as those appreciated by the eye. When converting the former into the latter, as must always

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be done, it should be remembered that one unit of blue, and one unit of red, and one unit of yellow when combined give one unit of black or neutral tint: and, further, that one unit of red and one unit of yellow together give one unit of orange. Now it is clear that in the above example the eye will only receive 0·4 units of black, because not more than 0·4 units can traverse all three colours. Since 0·4 units of red have now been required to produce the neutral tint, only 5·2 units of red remain. These, combined with an equal number of units of yellow, give 5·2 units of orange. Now 0·4 + 5·2 units of yellow have been used to produce the neutral tint and the orange, which when subtracted from the total 9·8 units of yellow, leave 4·2 yellow units remaining. Thus to obtain the visual colours from the glass units: treat the blue as black; subtract this from the red to obtain the orange; and subtract the red from the yellow to obtain the yellow. The two expressions thus found may be considered virtually as the two halves of an equation. As an example of the readings usually obtained the following colour measurements of two insects are taken from the protocols:

Insect	Wing	Standard glasses used				Visual colour		
		Red	Yellow	Blue		Black	Orange	Yellow
'17 C ♂ 9	Left fore	5·3	12·9	0·9	=	0·9	4·4	7·6
"	" hind	2·8	5·4	0·7	=	0·7	2·1	2·6
'17 C ♀ 44	Right fore	4·5	9·0	0·8	=	0·8	3·7	4·5
"	" hind	2·8	5·0	0·3	=	0·3	2·5	2·2

Throughout the paper all figures in square brackets refer to colour measurements. The first number is the orange value, the second Or. Yel.

number the yellow value, thus: [2·5 : 2·2]. As a rule, the black value, if any, was so small that it has been omitted.

III. METHOD OF PRESENTING RESULTS.

(a) *Curves showing the distributions of the colour-values (Figs. 15—25).*

It was found that the colours of the insects did not fade appreciably when they were kept in the dark. The colour of every insect was taken separately in the manner already described (see p. 213), the conditions of lighting, etc., being kept as constant as possible. With the readings obtained from a series of insects, curves to show the distribution of the colour-values can be constructed by plotting these values as ordinates, the abscissae being determined by the numerical positions of the individuals in the series, after the colour-values of all the insects have been

arranged in order of magnitude¹. Each abscissa then represents an individual insect.

The distributions which result for each family, or group of families, have been given in full at the end of the paper, as it was considered a more concise and accurate method of publishing the data than any form of table.

In each figure the heavy black line denotes the orange values. Orange is the dominating colour, but owing to the particular dyes used in making the coloured glasses, more yellow units are always required than red. This excess of yellow is much less important than the orange, since a considerable increase or decrease in it alters the colour tone much less than quite a small change in the red. It is nevertheless significant, so the values have been shown in each case by means of a small circle on the same perpendicular as the corresponding orange value. It will be seen at once that these yellow values do not follow the same order as the orange, and an oscillating curve results. This merely means that of two given oranges, the paler, i.e. the one containing the least red, may contain more yellow than the darker, or *vice versa*. For this reason it has been thought well to arrange the yellow values, also, in their order of magnitude. The resulting curve is shown by a line of crosses, but it must be remembered that any given cross does *not* necessarily refer to the same insect as the orange value on the same perpendicular. The yellow curve arranged in the same order as the orange has been called "yellow *a*," the yellow curve rearranged in its own order of magnitude "yellow *b*." Generally speaking it will be seen that the yellow values are in most cases approximately equal to the orange, so that the curve "yellow *b*" runs roughly parallel to the orange curve.

In the case of single families, the colour-values of the two parents have been printed in the margin in square brackets, beside the arrow which indicates, at the appropriate point along the colour scale, the orange value of each parent. Where more than one family is included the mean value of the parents has been given.

(b) *The frequency distributions* (Figs. 2—14).

Although the curves showing the distribution of the colour-values have been given chiefly to serve the purpose of a record, yet for the sake of convenience and simplicity, and in order to bring out the most salient

¹ This method has been suggested for anthropological studies by Eug. Dubois (*Man*, Vol. viii, June, 1908).

features, the colour-values of each family or group of families have been expressed as a percentage frequency distribution. Among other advantages this method tends to remove differences caused by the size of the group dealt with. The following method was adopted when converting one curve into the other.

The colour scale was divided into intervals of 0.4 units each (e.g. from 0.0 to 0.4, and 0.4 to 0.8, etc.). The number of individuals occurring in each colour interval was found, and the result divided by four, so that the percentages might all refer to the frequency in $\frac{1}{10}$ of a colour-unit. These average numbers of insects of any given colour-value were then plotted as ordinates, and the colour-values as abscissae. On account of certain errors mentioned below, an interval of 0.4 units was chosen, since this gave a reasonably smooth curve; though occasionally small oscillations appear, which probably have no significance.

The orange values are shown by the heavy line. Though of less importance the yellow values have been treated by the same method and are represented by a fine broken line. Roughly speaking it is seen to run parallel to the orange curve, as might be expected. As in the case of the distribution curves the colours of both parents have been shown in the margin below the arrow which represents their orange values.

(c) *Experimental and statistical errors.*

Whenever a frequency distribution rises to two maxima they correspond to those portions of the figures showing the distributions of the colour-values which are flattest, and which consequently contain the greatest number of insects of one colour. The points at which the frequency distributions reach a minimum (about 2.0—2.5) correspond to those portions of the curve showing the distribution of the colour-values where the fall is most rapid, and therefore to those points at which segregation appears to occur.

The question that must then be considered is whether the fall is real or whether it can be more easily attributed to the various sources of error. Errors due to technique, such as labelling, are, I think, insignificant. There remain those due to taking the colour measurements. The accuracy with which the readings can be taken varies considerably. A difference may be just discernible between two colours, one measuring 1.00 and the other 1.01 units, but no change will be appreciated between two other colours, one measuring 10.00 and the other 10.01 units. To be discernible the increment in the last case should be about 0.1

(though in practice it appears to be less), because the stimulus required to cause a change of sensation is always a definite fraction of the original stimulus. The paler colours can therefore be measured with greater accuracy than the darker. The chief error, however, is caused rather by the differences in the colour of the wing area selected for measurement, than by the inability of the eye to discriminate finer shades. As a rule the yellow colour becomes slightly paler towards the periphery of the wing. Therefore since exactly the same area cannot always again be found, the experimental error is increased by repeating the measurements. The following figures give some idea of the differences in the colour of an average specimen:

	Black	Orange	Yellow
Area round discoidal spot	—	3.2	3.6
„ between discoidal spot and central fascia	—	2.8	2.7
„ near hinder angle	—	2.7	2.9
„ near costa	—	2.6	2.7
„ near apex	—	2.3	2.0
Central orange fascia	0.2	5.0	6.0
Orange shoulder knot	0.8	4.5	5.7
Black discoidal spot	10.0	1.4	1.4

On account of this variability a practice was always made of selecting the large yellow area surrounding the discoidal spot, the more perfect wing of the two always being chosen. When this area was torn, or too badly rubbed, the nearest portion, and therefore the one most approaching the same colour, was selected. To avoid prejudice, all the readings were taken before the curves were constructed, and in most cases the readings were repeated twice. It was found from experience that at about the range in question there was a maximum experimental error of 0.1 colour-units. With regard to the question whether the data prove that the families and groups of families dealt with are really heterogeneous, and not single samples, I have shown the evidence to Mr Udny Yule. He has most kindly considered the matter and reports as follows: "As regards figures obtained in the $DR \times RR$ cross (Figs. 4 to 9) I do not think there can be any doubt that the data can be held to prove segregation. The gap is wide and well marked and occurs in the same position in several families or groups of families. In the case of the $DR \times DR$ cross (Figs. 10 to 13) so well marked a separation cannot be expected, as the second maximum does not rise to a sufficient height, and the distribution of the R 's only causes a comparatively slight hump on the tail of the distribution. A check on the result can however be obtained by building up the distribution that should be expected on the theoretical basis, showing that fair agreement is obtained." This check has been applied with fairly satisfactory results. In the case of the $DR \times DR$

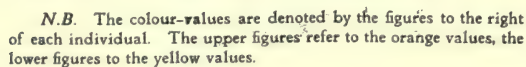
cross the fit is especially good, the second maximum being no higher on the theoretical basis than on the experimental. In the case of the $DR \times RR$ cross, the maxima, especially the second, though no higher, are shifted somewhat to the right. This is no doubt due to the fact that the average colour-values of the parents in the former case are considerably higher than the average value of the yellow grandparents of the $DR \times RR$ cross, since the yellowest insects were selected for breeding. It seems to be fairly clear therefore, from the foregoing considerations, that a considerable degree of segregation actually takes place.

(d) *Colour of the hind wings, and effects of desaturation and sex.*

In every case the hind wings are paler than the fore wings, and in a large number of insects separate colour readings were taken of them. In many of the medium and paler specimens the hind wings are quite white, about $[1.0:0.7]^1$. In the case of one family, '17C (see Figs. 5 and 18) the orange values of the hind wings have been shown as a curve, but as no special importance was attached to these figures, elsewhere they have been omitted. On the curve (Fig. 18) it is seen how the paler insects uniformly have white hind wings, irrespective of small differences of colour in the fore wings.

Mention has already been made of the fact that many of the colour measurements required the inclusion of a small quantity of black, before exact equivalence could be obtained. This amount is very variable, and occurs most irregularly in any series of insects. The highest values are always among the deepest yellows, but even among these there are some devoid of all black pigment. Usually the pale yellow forms have no black, but white insects generally require a small quantity. The deepest yellows no doubt actually contain a little melanic pigment, which under the microscope appears as an almost imperceptible mottling upon the scales. In the white insects the greyish colour of the surface is caused rather by displacement or removal of the scales, than by the presence of melanic pigment. These injuries are more apparent in white insects than in yellow ones. Owing to the misleading effects of injury, and to this lack of uniformity in the black values, they were not considered of sufficient importance to record in every case. In family '17C, however (see Fig. 18), these values have been indicated by means

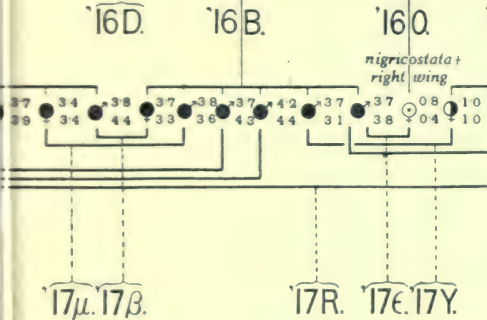
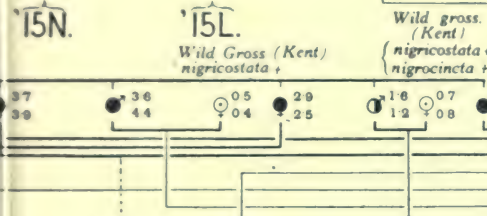
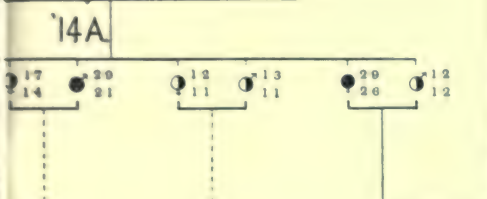
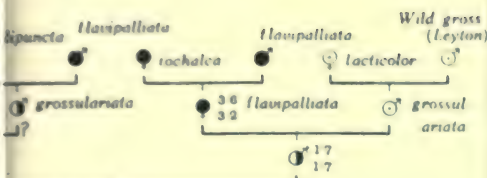
¹ N.B. All figures in square brackets refer to colour measurements: the first number denotes the orange value, the second number the yellow value. The black values have been omitted.



● = Homozygous for yellow, i i.
 ⊗ = The same combined with *lacticolor*.

II.

MA STANER.



for the inhibition of yellow (white) II. combined with lacticolor.

1905

1906

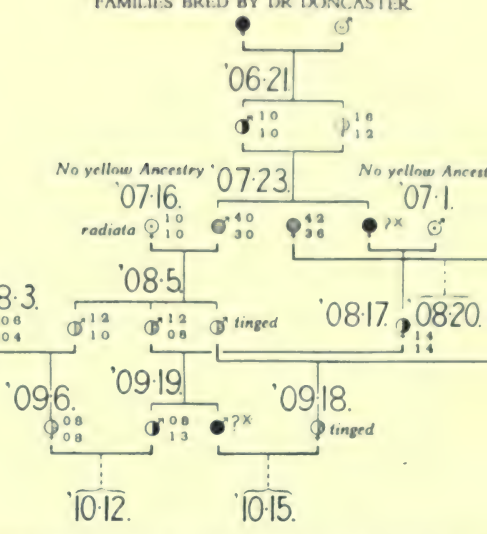
1907

1908

1909

1910

FAMILIES BRED BY DR. DONCASTER.



* The colour of these insects could not be measured, but record of their emergence makes it probable they were homozygous yellow.

'15P. Wild gross. (Kent) nigricostata + nigrocincta + nigricostata + Wild gross. (Kent)

'16T.

'16P.

'16H.

'17E.

'17P.

'17O.

● - Heterozygous for the inhibition of yellow. II. (Very pale yellow, or white)
 ○ - The same combined with lacticolor.

of diamond-shaped dots under the orange value of the appropriate insect.

In preparing the curves to show the distribution of the colour readings, the sexes were at first kept separate. In family '16A (see Fig. 21) the females have been shown alone, just below the curve which comprises both sexes. Possibly there is a slight tendency for the females to be paler than the males, and in the family in question it can be seen that rather a larger proportion than usual of the paler forms are female. On the whole, however, the sex appears to have little effect. Accordingly, elsewhere individuals of both sexes have been treated together. Further, in the crosses, the sex of the yellow parent appears to have no effect on the offspring.

(c) *Table of families, and pedigree.*

In many of the families there occur some of the varieties which are described on pp. 220—223. Their names have been given below the curves showing the distribution of the colour-values at the end of the paper, and to the right of the name is shown the sex and the number of the insect in the curve. These numbers refer of course to the figures along the base line of the curve, so that any individual can be looked out, and the colour-value read off from the curves. For instance in family '16G (see Fig. 24) the variety *violacea* occurs. It is marked ♂ 24. From the colour-values of the curve it may be seen that the 24th insect is [1.0:0.4]. The colour-value of any other variation may be looked up in the same way. When, however, several families are combined in a single curve, a difficulty arises. There is nothing to show in a compound curve which insects belong to which family, and therefore it cannot be found from inspection which individuals in a given family do *not* show the particular variation under consideration.

Reference to Table I will give this information, as the sex and number of every individual in each family has been there tabulated. The gametic constitution of every insect used for breeding, as indicated by the offspring, is shown by the symbols employed in the pedigree (see Table II). The sex, colour-value and any other details known are also given, and the colour-values of all the offspring may be determined by reference to Table I and the appropriate figure. All the details subsequent to 1914 have been composed from my own data. For records previous to 1914 I am indebted to the Rev. G. H. Raynor, who also gave me my original material. A few of the insects bred before 1914

were sent to me, and the colour was measured in the usual way. In the other cases, although the colour could not be determined, it is clear from the description whether the insect was a homozygous yellow, but it is not always so clear whether it was a hybrid or a type. In these circumstances the probable constitution of the insect has been indicated by the symbol, and a note of interrogation has been added to show that the question is doubtful. The pedigree of six families of set insects, kindly given to me by Dr Doncaster, has been inset on Table II.

IV. DESCRIPTION OF CERTAIN VARIETIES.

As is well known, *A. grossulariata* is an extraordinarily variable species, and Mr Raynor has named and described a number of its aberrations¹. His nomenclature therefore has been used whenever possible, but in two cases I have been reluctantly forced to adopt new names to denote slight variations for which no published name could be found. The following descriptions include all the variations which have been met with in the course of the experiments, but it is by no means a complete list.

Var. *chrysostrata*.

(See Plate IX, Nos. 31 to 42.) This name is given to the variety of *lacticolor*, which is entirely suffused with yellow.

Var. *flavi-* or *albipalliata*.

(See Plate IX, Nos. 1, 7 and 19.) The black markings are absent from a broad area between the black basal blotch and the discoidal spot, which gives a white (or yellow) mantle about that area of the insect. This variety only occurred among the parents of my strain, and not much information could be derived from Mr Raynor's records as to the nature of the inheritance of this character. In four families both parents of which were *flavi-* or *albipalliata* less than 7 per cent. of the offspring showed the variation.

Var. *fulvapicata*.

(See Plate IX, Nos. 3 and 28.) The orange colour of the central band is continued to the apex of the wing, and the black marking at the apex is obsolete. The incomplete development of the scales at the apex, occasionally the result of inbreeding, may cause a superficial resemblance to this variety.

¹ "Notes on *Abraxas Grossulariata* and how to rear it," *Entomologist's Record*, Vol. xiv. p. 321, 1902 and Vol. xv. p. 8, 1903.

Var. impunctifasciata.

(See Plate IX, No. 24.) This variety has not been previously described. The black band of spots on the outer side of the orange fascia may be completely or partially obsolete. It seems that this variety should have a close connection with the preceding one, but there is no evidence from which to decide. Either the portion of the band near the inner margin or the central portion may be obsolete, without the apex of the wing being affected. In order to distinguish between the different amounts of black present in the band of these insects, in Table II and elsewhere they have been qualified in the following way:

- impunctifasciata* + signifies that about half the band is obsolete.
 „ ++ signifies that three quarters of the band is obsolete.
 „ . +++ signifies that the band is completely obsolete, or only traces of it present.

(a) *The Melanic Series.*

A certain number of varieties may be collected under this heading. They consist of variations in the size and position of the black markings on the wings and body, and of a suffusion of dark pigment over the whole wing surface. For convenience they will be considered together. In several cases, although different names have been given to the variations, one often only denotes a more advanced stage of melanism than the other. The furthest stage of melanism is found in var. *varleyata* (Porritt)¹. I am at present investigating the relationship of this variety to the type as well as to *lacticolor*.

Var. radiata.

(See Plate IX, No. 58.) Marginal spots radiated, generally on the fore wings only. This variety may of course occur in *grossulariata* as well as in *lacticolor*.

Var. nigricostata.

(See Plate IX, Nos. 16, 22 and 28. No. 44 also shows traces of a black stripe.) The black stripe may extend from the shoulder knot either to the discoidal spot or throughout the whole length of the costa. Sometimes the black marking below the discoidal spot is confluent, so that a

¹ An excellent coloured illustration of var. *varleyata* (Porritt) is given by W. Bowater, Plate XXVII, No. 45, *Journal of Genetics*, Vol. III, 1914, p. 299.

second black stripe runs from the middle of the shoulder knot towards the third marginal spot, which it may or may not reach, leaving one or two small wedge-shaped areas of yellow or white in the centre of the wing. (See Plate IX, No. 16.) The different lengths of this black stripe have been indicated in the following way:

- nigricostata* signifies costa black as far as discoidal spot.
- „ + signifies costa black as far as orange fascia.
- „ ++ signifies costa black as far as the apex of the wing.
- „ +++ signifies second black stripe running more or less completely across the wing.

Var. hazeleighensis.

(See Plate IX, No. 30.) Fore wings almost filled with black except for small white specks in the middle of the costal margin. The hind wings are sometimes banded, but are often unaffected. This variety frequently occurs in the cross *varleyata* × *grossulariata*.

Var. nigrocincta.

(See Plate IX, Nos. 16 and 30.) This variety has not been previously described. It is caused by the dorsal spots being extended round the body so as to form black rings. In some specimens the body may be almost entirely black. The variety seems sometimes to be correlated with the amount of black pigment on the wings, but this is not always the case, because the dorsal spots in many specimens of *varleyata* are quite normal¹.

The degree of pigmentation is indicated in the following way:

- nigrocincta* + signifies black rings incomplete.
- „ ++ signifies black rings complete.
- „ +++ signifies dorsal area entirely black.

In some insects the black rings are only found on certain abdominal segments. In these cases the correct segments are indicated in brackets, just below the variation on the distribution figures.

Var. violacea and *var. semiviolacea.*

(See Plate IX, Nos. 11 and 27.) Either all four wings or only one pair may be suffused with a purplish brown bloom that gives the insect a scorched appearance. Under the microscope the pigment is seen to be purple, and evenly distributed over the surface of the scales, which have a faintly mottled appearance. Otherwise it strongly resembles the suffusion of *iochalca* (*vide infra*).

¹ Compare the insect illustrated by Bowater (see note p. 221).

Var. nigrosparsata.

(See Plate IX, No. 29.) All the wings are finely speckled with black, which gives the insect a sooty appearance, that extends over the orange fascia. It is said that this variation is not inherited, and Mr Porritt has told me that he failed to breed a greater percentage of specimens from *nigrosparsata* ♀ × *nigrosparsata* ♂ than he was able to obtain from wild larvae collected in the same neighbourhood. Mr Raynor has also had a very similar experience. I once obtained a single specimen of *nigrosparsata* ♂ from larvae collected in Kent, which was paired to a rather dark female from the same locality: out of 12 offspring only one ♀ showed a very faint trace of melanic suffusion.

Var. iochalca.

(See Plate IX, No. 60.) This variety bears a certain relationship to the preceding one and to *violacea*, in that they all have a melanic suffusion which extends over the surface of the wing. The difference in appearance is however most striking, as may be seen by reference to Plate IX. In *iochalca* the wings are suffused with a peculiar metallic hue due to the addition of black pigment to the scales of the yellow variety of *lacticolor* (i.e. *chrysostrata*), which causes a desaturation of the yellow colour. In *nigrosparsata* on the other hand, the suffusion is more intense and often has a mottled appearance, especially when observed through a lens. It is true that the specimen illustrated (No. 29) has a white and not a yellow ground. Nevertheless, when *nigrosparsata* is combined with *lutea*, the difference is if anything more striking.

(b) Distribution of black pigment in suffused varieties.

A yellow insect showing this suffusion was bred too late to be included in the plate. A separate illustration, however (see Plate X), shows the difference between the deposition of the melanic pigment in the yellow variety of *nigrosparsata*, and in a specimen of *iochalca* as they appear under the microscope. The portion of the fore wing of the *nigrosparsata* illustrated is much closer to the costa than that of the *iochalca*, and consequently the scales are smaller and narrower. A comparison of the two figures shows that the scales of *iochalca* (see Plate X, Fig. 1) are a uniform buff, the black pigment being rather more abundant near the base of the scales than at the tip. For this reason the pigmentation is somewhat obscured, because the darkest portion is always covered by the pale overlapping points of the scales in the row preceding it. The wing

of *nigrosparata* (see Plate X, Fig. 2) on the other hand is seen to have the black pigment concentrated in certain scales, the majority of yellow scales being practically devoid of dark pigment. In contrast to the case of *iochalca* however, the dark pigment appears to be concentrated in the tip of the scale rather than at the base, which accentuates the mottled effect already caused by the localisation of the black pigment in a few scales. The few grey scales of *iochalca* scarcely affect the colour.

Mr Raynor has kindly given me data concerning several pairings with *iochalca*. It appears to be recessive to *A. grossulariata* and to breed true when mated together. A pairing between *iochalca* ♂ and *iochalca* ♀ gave 13 females and 6 males, all *iochalca*. Another family of *iochalca* ♂ × *albipalliata* ♀ gave 5 females and 1 male *iochalca*, and 12 males and 15 females of other varieties, 14 of these females being *lacticolor*. It must of course be remembered that the inheritance of this variety may be complicated by the fact that it probably never occurs except in combination with *lacticolor*. Experiments are in progress with a strain of this variety kindly given to me by Mr Raynor.

Var. *cupreofasciata*.

(See Plate IX, No. 59.) This variety has the melanic suffusion of *iochalca*, restricted to the orange fascia and shoulder knot. It can be seen that the fascia has exactly the same appearance as that in No. 60, Plate IX, and may be contrasted with the bright yellow fascia of the ordinary *lacticolor* insects (Nos. 55 and 56). Mr Raynor tells me that insects of the variety *cupreofasciata* are invariably descended from *iochalca* ancestry.

V. THE YELLOW PIGMENT.

The cause of the difference in the intensity of the yellow pigment in the pale and the deep varieties is a problem of considerable importance, since it may possibly give a clue as to the nature of the factors which determine the varying shades of colour. There are several possible conditions which might account for these colour variations.

(1) The pigment itself may vary in constitution, and consequently in colour.

(2) The concentration of the pigment, which may be either diffused or in the form of granules, may be increased in the deeper varieties.

If the composition of the pigment varies the change need not be very fundamental, but might merely consist in an alteration in the

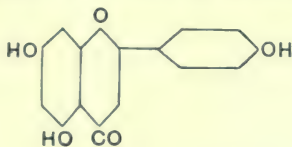
position or number of certain chemical groups¹. If the change is quantitative and the pigment is diffused, the amount "in solution" may be increased, or the pigment may become aggregated in small granules or masses. The orange-red pigments of the Pieridae are all yellow in aqueous solution, the orange colour being probably due to increased concentration². The pale yellow butterfly *Colias hyale*³ is said to contain mostly diffused pigment, whereas the more deeply coloured *C. edusa* contains granular masses of pigment between the walls of each scale.

(3) A similar deepening of colour might be produced by an increase in the number of scales, i.e. several overlapping layers of scales would give a deeper shade than a single layer.

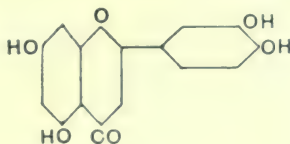
(4) Finally, the position of the pigment within the scale itself might affect the colour, which would depend on the proximity of the pigment to the upper surface, e.g. it might be chiefly in the upper or lower membrane of the scale, or it might be between the two⁴.

Clearly a correct decision of these questions is of the utmost importance, for if the pigment varies quantitatively and not qualitatively, the factor or factors which control the amount must be capable of acting in a quantitative manner upon the pigment-producing mechanism. Now this mechanism, in many if not in all cases, consists of an enzyme (usually oxidising) which acts upon a colourless chromogen. It is difficult to see how the addition or subtraction of a factor—a change

¹ In the yellow flower pigments for example the pale yellow flavone Apigenin



becomes the much more deeply coloured pigment Luteolin,



i.e. an extra hydroxyl group appears in the side ring.

² F. G. Hopkins, *Phil. Trans.* Vol. 186, B, p. 678, 1895.

³ W. Geest, *Zs. wiss. Insektenbiol.* Berlin, Vol. iv. p. 162, 1906.

⁴ A case of dilution of colour owing to localisation of pigment occurs in the hairs of black and blue mice and rabbits. H. Onslow, *Proc. Roy. Soc. B*, Vol. 89, p. 56, 1915.

which cannot but be called qualitative—can cause a quantitative difference in the amount of pigment deposited by an enzyme-substrate system. The only factors that are capable of influencing such a system quantitatively would appear to be those such as time, temperature, mass action, hydrogen ion concentration, etc. Increased pigmentation may of course be produced by the removal or suppression of inhibitors, but if there are a great variety of shades to be accounted for, as in the case of *lutea*, this may lead to the employment of an absurd number of inhibitors before the phenomena can be accounted for.

Distribution of the pigment in scales of insects of varying shades of yellow.

A preliminary microscopical examination of a number of insects showed that the colour was by no means evenly distributed throughout the wing, but that patches of pale and more deeply coloured scales were often intermingled. Even in a single scale the pigment was by no means uniformly deposited. On the whole, however, it can be said that in the orange varieties the scales are a much deeper yellow than in paler specimens. This observation was confirmed by examining the scales by transmitted light. To investigate the condition of the pigment more carefully a number of sections were made of wings from individuals of various shades. As is well known, a scale is a flattened sac and appears in section as shown in Plate X, Figs. 3, 4 and 5. With considerable difficulty thin uniform sections with an average thickness of about 2 to 3 μ were obtained. An inspection of these showed at once that the pigment was present "in solution" within the chitin, and in no case could any granular pigment be found even in the deepest yellow insects. A scale from a pale insect is shown (Fig. 5) having a value of about [1.8 : 2.0]. Very pale and white specimens have so little pigment that thin sections are practically invisible when mounted in balsam. A scale from a moderately yellow insect (Fig. 4) with a colour-value of about [2.8 : 3.0] is also shown. This section is cut near the base of the scale, not far from the root, as is indicated by the considerable increase in the distance between the two walls at the centre. The deepest orange section (Fig. 3) came from an insect with a colour-value of about [4.0 : 4.0]. Clearly the pigment is diffused throughout the chitin, and therefore any increase in colour must be caused, either by a qualitative difference in the pigment, or by an increase in its concentration.

VI. THE ORIGINAL MATERIAL.

I am indebted to the kindness of the Rev. G. H. Raynor for the material with which the breeding experiments were carried out. This strain of *lutea* originated in 1904 with two pale insects bred from wild larvae collected in the neighbourhood of Warrington. These insects were shown to me: the ♂ had a value of about [1·7 : 1·5], and the ♀ a value of about [1·0 : 1·3], being only just tinged with yellow.

Dr Doncaster most generously gave me six families of set insects bred by him between 1906-1910, and with his permission, I have included the data from these families with my own. Each family bred by Dr Doncaster has been marked with an asterisk wherever it occurs.

My original material consisted of two batches of ova sent by Mr Raynor in 1914. The female parent of one family ('14 B), was a wild insect previously fertilised by a yellow male. The wild ♀ was captured in Milltown Park, Dublin, the locality in which the "Q variety," described by the Rev. J. M. Woodlock¹, was found. All the F_1 generation was therefore heterozygous for yellow. The parents of the other family ('14 A) were described as a "pair of yellows." One of these yellows which I was able to examine had a low colour-value, i.e. [1·7 : 1·7]. As a rule, homozygous yellows, as can be seen from Fig. 16, have a much higher value, at least above [2·4 : 2·4]. Moreover, it will be seen from the pedigree that the male in question was bred from a *flavipalliata* ♀ × *grossulariata* ♂. Consequently it is much more probable that the insect was a yellowish heterozygote, than a homozygous yellow, and therefore the family '14 A is $DR \times RR$, and not $RR \times RR$ as was first thought. This view is supported by the fact that of the total offspring (10 ♂♂ and 12 ♀♀) 11 had an orange value greater than 2·6, and 11 an orange value less than 1·6. The material has therefore been treated in accordance with this supposition.

The larvae were reared with the usual precautions, great care being taken with the labelling, and every endeavour made to avoid introducing either eggs or very young larvae from one box to another. It seems almost impossible, however, to prevent a larva that has escaped from reappearing occasionally amongst the food when another brood is being dealt with. No doubt a small percentage of errors may be attributed to this cause, as for instance the appearance of some of the palest individuals in the *lutea* × *lutea* matings (see Fig. 16). In order to avoid the ill-effects of in-breeding, type insects were obtained for

¹ J. M. Woodlock, *Journal of Genetics*, Vol. v. p. 183, 1916.

the purpose of crossing, by means of larvae or pupae, from Cambridge, Kent and Yorkshire. Notwithstanding this precaution and the fact that the species resists in-breeding very well, all ill-effects were not avoided. In 1916-1917 many of the females became sterile, and the families of others were very small. Dysentery was also severe in 1916 and 1917, as may be seen from the small *lutea* \times *lutea* families in the latter year.

VII. BREEDING RESULTS.

The frequency distributions (Figs. 2—14) have been constructed from the curves showing the distribution of the colour-values (Figs. 15—25) in the manner described on p. 215. The orange colour-values of the parents have been marked with arrows on the scale of colour-units at the bottom of the figures, and the full colour-value has been printed in the margin. When several families are combined in one curve, the arrows indicate the mean orange value of the parents. When both parents are of the same gametic constitution, one arrow refers to the male and one to the female parent. In the case of a cross containing several families, the arrows refer to the varieties of the parents regardless of their sex. The names of all the families included in any curve are printed on the figure, together with the year in which the eggs were laid, thus:—'16 M, '17 E, etc.

(a) Var. *lutea* \times *A. grossulariata*.

For the sake of clearness it is well to divide the colour scale into five arbitrary classes, corresponding to the five columns of insects in Plate IX. This division of the colour scale in both types of distribution curves helps to bring the phenomenon of segregation into evidence. The white or lowest class includes colour-values, from 0.0-0.8, of the type insects (Plate IX, Nos. 25 and 26). The second class, from 0.8-1.6, are the slightly tinged individuals heterozygous for yellow (Plate IX, Nos. 19 to 24). The third class rather deeper in colour, from 1.6-2.4, is as a rule also heterozygous for yellow (Plate IX, Nos. 13 to 18). It does not contain more than a very few individuals, because it embraces just that part of the scale where segregation takes place, and where the frequency is therefore lowest. The fourth and fifth classes comprise the homozygous yellows, from 2.4-3.2, and from 3.2-4.0, or above (Plate IX, Nos. 1 to 12).

The F_1 heterozygotes *lutea* \times *gross.* cross can usually be distinguished from the type by slight differences of form or colour. Over a thousand

type specimens, collected from various localities, have been examined, and in no insect did the colour-value exceed [1.1 : 0.3], whereas the great majority were [0.7 : 0.6] or less.

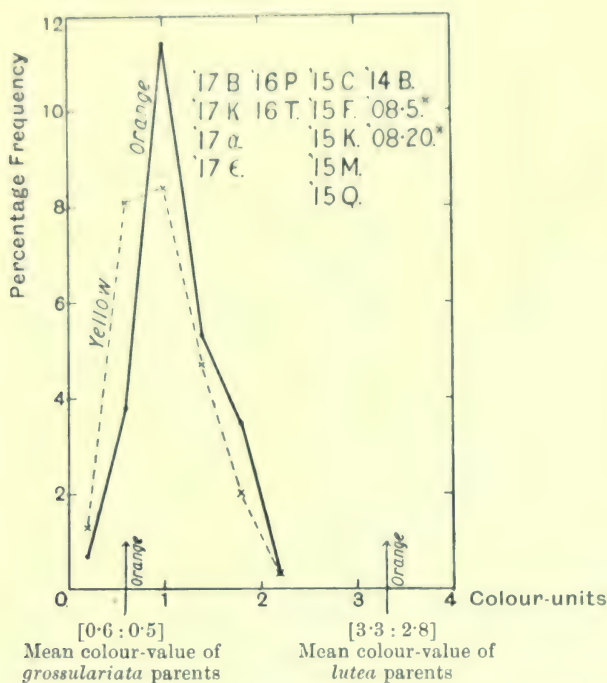


Fig. 2. (Cf. Fig. 15.) Curve showing frequency distribution of the orange and yellow colour-values of the offspring from 14 pairings of *lutea* × *grossulariata*.

All the families resulting from pairings of *lutea* × *grossulariata* are small and have been combined in a single curve (see Fig. 15). The mean orange value of the yellow parents was 3.3 or nearly six times as yellow as the mean value of the type parents, which was 0.6. The frequency distribution (Fig. 2) reaches a maximum at about 1.0, showing that the factor for yellowness is not completely recessive, because the majority of the offspring are more deeply coloured than their type parents. However the orange colour represented by 1.0 unit of orange is a very pale cream, rather paler than insects Nos. 20 and 21, Plate IX. It was found to make no difference in the offspring whether the male parent was a *grossulariata* or a *lutea*, so the reciprocal matings have been combined in one curve. They can be easily separated, however, by reference to the pedigree and Table I. The curve though rather narrow is not unlike an ordinary frequency distribution. The range of

variation is small, though the slight broadening of the curve, just before a colour-value of 2.0, shows that a small percentage of the offspring are very perceptibly tinged with yellow. The result of mating one of these rather yellow hybrids with a type insect will be seen in section (e), p. 239. The broken line denotes the curve formed by the excess of yellow in the colour measurements, and it runs roughly parallel to the orange curve.

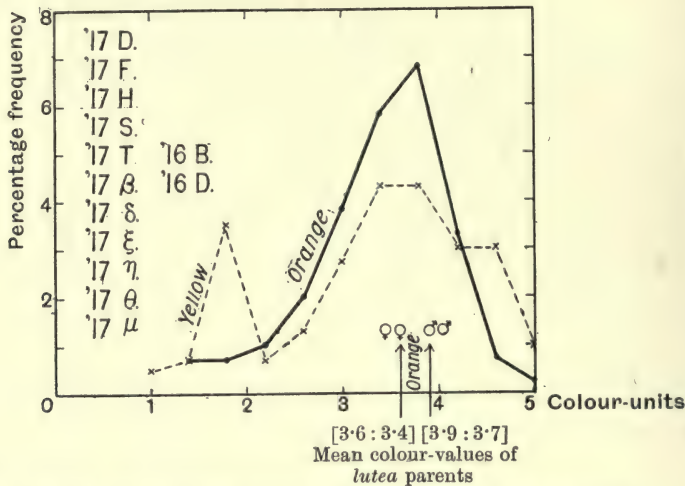


Fig. 3. (Cf. Fig. 16.) Curve showing frequency distribution of the orange and yellow colour-values from 13 pairings of *lutea* × *lutea*.

(b) *Var. lutea* × *var. lutea*.

This curve (Fig. 3) is again composed of a number of small families, and though asymmetrical, it is more like a normal frequency curve than Fig. 2. It reaches a maximum immediately above the arrows indicating the mean orange value of the respective parents. The base of the curve is fairly broad showing that the range of variation is considerable. Reference to Fig. 16 shows that about 10 insects have an orange value below 2.5. Although some of the parents had a low value, as may be seen from the pedigree, these offspring are unusually pale, and there can be little doubt that some of them are due to experimental errors, i.e. of labelling or while feeding. The general appearance of the black pattern on one or two of them is quite dissimilar to that of the other members of the family, and these no doubt have strayed from elsewhere. This explanation cannot account either for all the pale individuals in this cross, or for the deepest yellows in the F_1 generation.

The broken line indicating the excess yellow in Fig. 3 *et seq.* runs as usual closely parallel to the orange curve, except for a sharp peak in the pale region, which is no doubt only an irregularity caused by an insufficiency of numbers.

(c) *Hybrid*¹ (*lutea* × *gross.*) × var. *lutea*.

A group of small families, none of them large enough to be treated separately, has been first dealt with (Fig. 4). As would be expected in this type of mating (*DR* × *RR*) the offspring segregate into two separate classes. The hybrid insects have a value of 0.8-2.4, and the yellow insects one of 2.4 or above. The curve reaches a minimum at 2.2, and the two maxima occur at just about the mean orange value of the yellow and hybrid parents respectively. The areas of the two curves should be equal, and should represent the number of individuals in each class: actual measurement shows that the percentage of the dominant form is 54, and that of the recessive form 46. The yellow values show a rather definite decline for the *lutea* homozygotes.

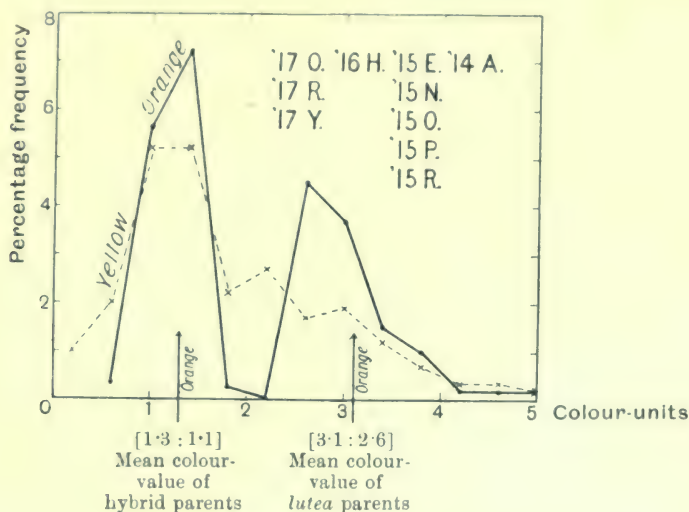


Fig. 4. (Cf. Fig. 17.) Curve showing frequency distribution of the orange and yellow colour-values of the offspring from 10 pairings of hybrid (*lutea* × *gross.*) × *lutea*.

¹ Although the word "hybrid" strictly applies to species-crosses, it has been retained instead of the preferable term "cross-bred," since it was too late to make alterations in the figures at the end of the paper, where the first term had unfortunately been printed.

Figs. 5, 6, 7 and 8 show the curves given by four separate families in which the offspring were numerous enough to be treated by themselves. The essential features are much the same as in the last case. In family '17 C (Fig. 5) the *DR* individuals are indeed slightly darker than the ♀ parent (hybrid) whereas the *RR* insects are nearly all paler than the ♂ parent (*lutea*) which was very deep [4.3 : 4.9]. Two separate curves have been added to this figure to indicate the orange values of the hind wings, and the black values of the fore wings (see p. 218).

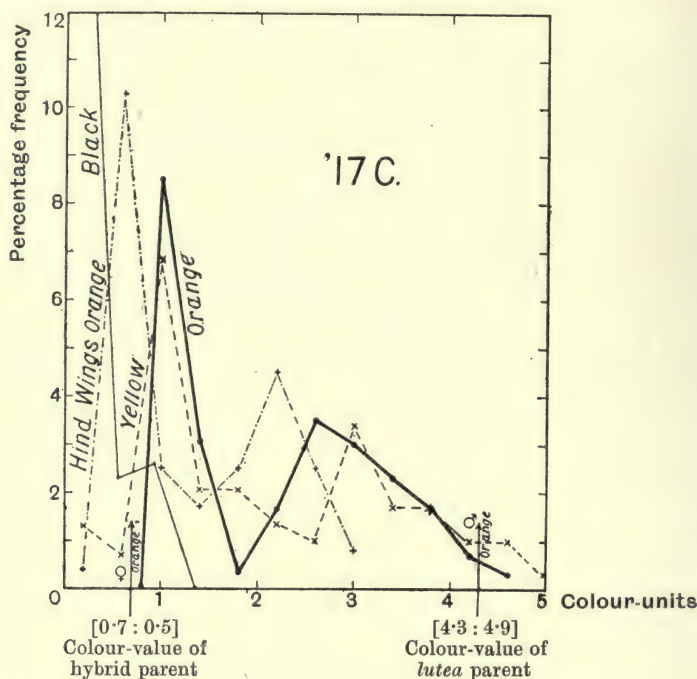


Fig. 5. (Of. Fig. 18.) Curve showing the frequency distribution of the orange and yellow colour-values of family '17 C, *lutea* × hybrid (*lutea* × *gross.*).

The orange colour of the hind wings is also shown by means of a dot-and-dash line. The small quantities of black which occur so irregularly in the fore wings are shown by means of a thin continuous line.

Family '16 E (see Fig. 6) shows a somewhat similar curve; the yellow ♂ parent [2.9 : 2.1] was not quite so dark as in the last figure, and the ♀ parent unfortunately was lost. However a record exists saying that it was rather deeply coloured for a hybrid: the orange colour-value being probably not less than 1.7. The result seems to be a certain increase in the number of yellows. The double maximum

seen at a value of about 3.0 is probably only due to the small number of individuals available.

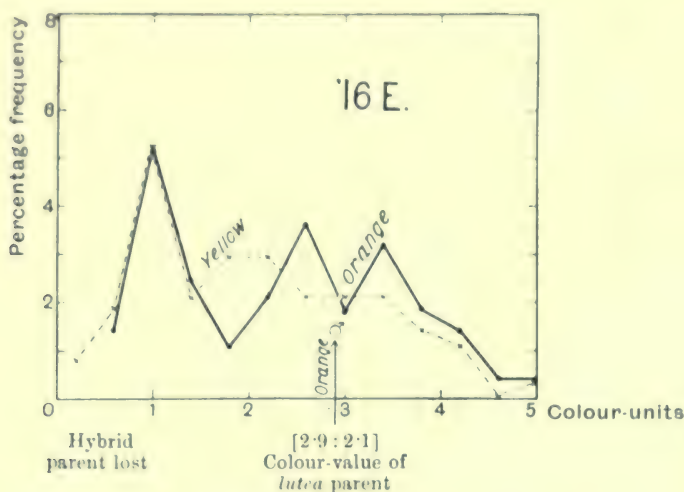


Fig. 6. (Cf. Fig. 19.) Curve showing frequency distribution of the orange and yellow colour-values of family '16 E, hybrid (*lutea* \times *gross.*) \times *lutea*. The φ parent (hybrid) was unfortunately lost.

Family '10.15*' (Fig. 7) shows almost exactly the same principal features as the preceding curve. Segregation appears to occur at the same point, and the homozygous yellows are again somewhat in excess,

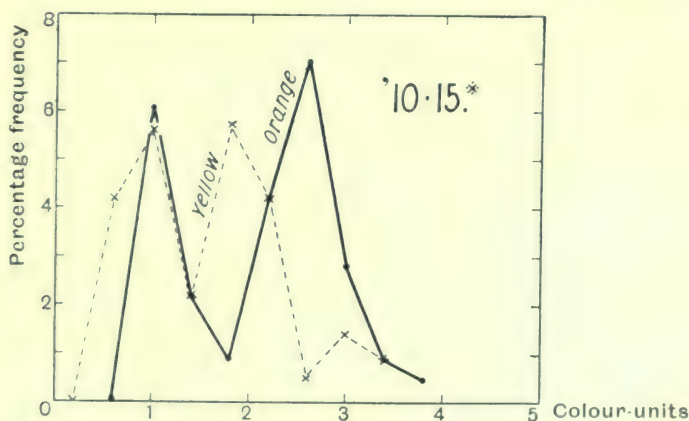


Fig. 7. (Cf. Fig. 20.) Curve showing frequency distribution of the orange and yellow colour-values of family '10.15*, hybrid (*lutea* \times *gross.*) \times *lutea*. Parents missing.

* Bred by Dr Doncaster.

but unfortunately the parental values cannot be shown as the insects were unobtainable.

Family '16 A (Fig. 8) shows yet another cross of the same nature. The yellow parent is again very dark, and the homozygous yellows are in excess of the hybrids. In this family the females have been kept divided from the males, and the orange colour-values of their fore wings

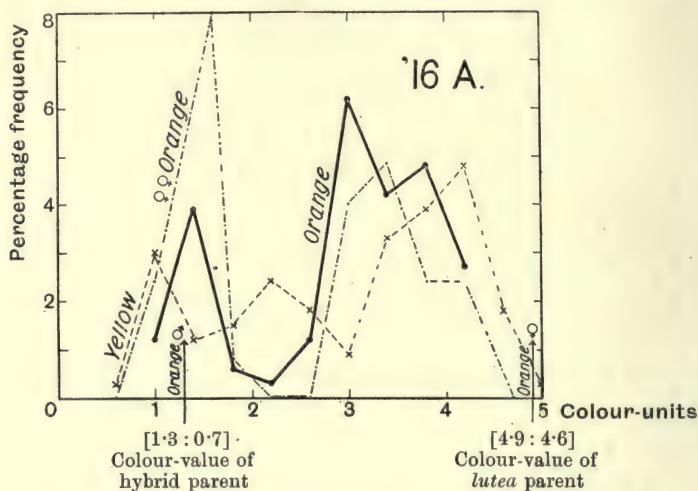


Fig. 8. (Cf. Fig. 21.) Curve showing frequency distribution of the orange and yellow colour-values of '16 A, hybrid (*lutea* × *gross.*) × *lutea*.

The orange values of the females alone are shown by means of a dot-and-dash curve.

have been shown by a line of dots and dashes, which differs in no essential from the same curve for both sexes (see p. 219). Reference to Fig. 21 shows that the excess of yellows over hybrids is greater than in the three preceding cases, there being at least 64 yellows to 20 hybrids, or a ratio of 76 : 24 (per cent.).

Now supposing the yellow colour develops in the variety *lutea* owing to the loss of an inhibitor *I*, from the white type *II*, then this excess of yellows might of course be accounted for on the hypothesis of another factor *X*, a deepener, which has no visible effect upon yellow *ii*, but which turns the pale hybrids *Ii* into deep yellows. If for instance the yellow parent of '16 A had been *Xx*, then half the hybrid offspring would have been *IiXx*, and half *Iixx*. Those carrying *X* and *I* would be changed into deep yellows, and the result would be 75 per cent. deep yellows, and 25 per cent. pale yellows or whites. The ratio observed in this family ('16 A) is very close to the result expected on this hypothesis.

In the three preceding families the yellows have always been in excess. The result of combining these three families is to make the ratio of the recessive form to the dominant 68:32 (per cent.), a somewhat lower proportion of yellows than in 16 A. In Fig. 4 the proportion of yellows to hybrids was about 46:54 (per cent.), but the evidence derived from the four large families of about 80 insects each, is undoubtedly more valuable than that derived from a number of small ones. Further the offspring from all pairings of the type $DR \times RR$ have been combined in one curve, Fig. 9, which resembles the five preceding curves in most essential points; and results in a ratio of 41:59 (per cent.). But as a matter of fact there are not sufficient data from which to draw a definite conclusion, especially as no confirmatory evidence is forthcoming from the other crosses.

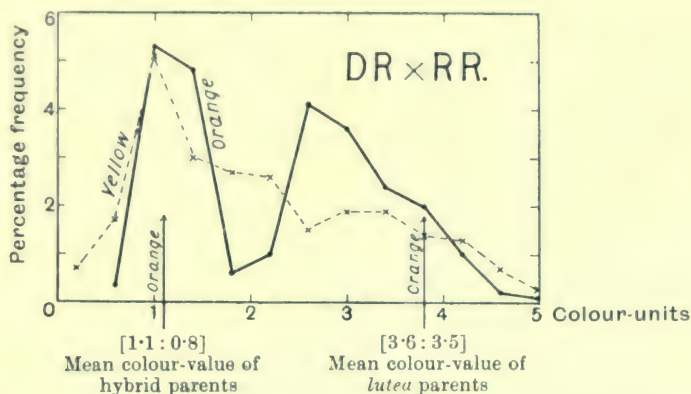


Fig. 9. Curve showing frequency distribution of the orange and yellow colour-values of the offspring from all the hybrid (*lutea* \times *gross.*) \times *lutea* families shown in Figs. 4, 5, 6, 7, and 8.

(d) *Hybrid (lutea* \times *gross.)* \times *hybrid (lutea* \times *gross.).*

The frequency distribution shown in Fig. 10 is composed of a number of small families of this cross, none of which were large enough to be treated by themselves.

If the heterozygous yellows, which are often distinguishable from the white type by their pale yellow colour, were really to form a distinct class to themselves there should be three maxima in Fig. 10. Clearly there are only two, showing that the pale yellow heterozygous class passes by insensible changes into the pure white DD class with an orange value of less than 0.8. It should however be carefully noted in Fig. 22 that there are 48 insects or 24 per cent. with a colour value of 0.8 and

under, whereas in Fig. 17 there are about 1 per cent. But since the maximum frequency for heterozygous yellows (about 1.0) lies so close to that of the white type insects (about 0.6 or 0.7), the ability to distinguish colours is not sensitive enough to permit of the two maxima being

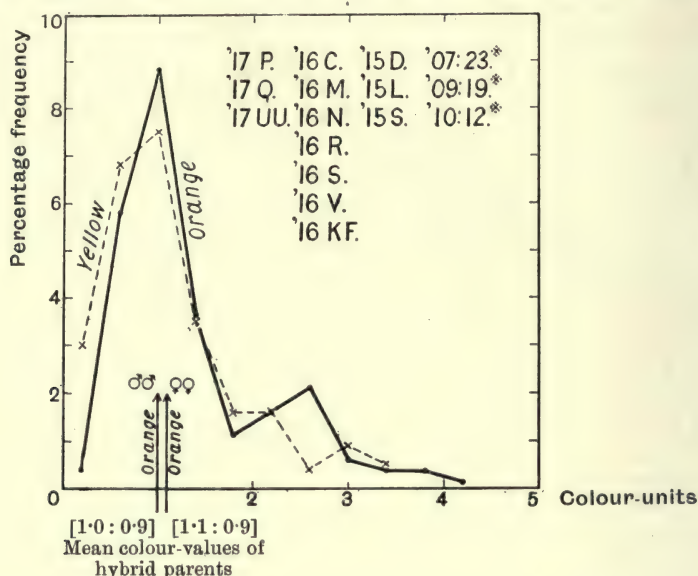


Fig. 10. (Cf. Fig. 22.) Curve showing frequency distribution of the orange and yellow colour-values of the offspring from 16 pairings of hybrid (*lutea* × *gross.*) × hybrid (*lutea* × *gross.*).

separated. Segregation between the heterozygous and pure yellows is distinct, though the frequency of the yellows does not reach a very pronounced maximum. The expected ratio, 25:75, is in this case exactly the observed ratio. It must not be forgotten that the length of the colour scale occupied by the yellow class is as great as that covered by both the type and the heterozygous classes, but it is the area of the curve not the height which corresponds to the number of individuals it contains. Some of the extracted yellows are very deep in colour, almost as dark as a pure-bred yellow, but the average orange colour-value (about 3.0) is rather less than what it is for pure yellows (about 3.6).

Figs. 11 and 12 are made from two fairly large families of the same type of cross. Fig. 11 is in most details similar to Fig. 10 except that both the yellow and orange curves rise to rather a sharper second maximum. Fig. 12 is not so characteristic because the family is only a

very small one. As before, Figs. 10, 11 and 12 have been combined and the result is shown in Fig. 13.

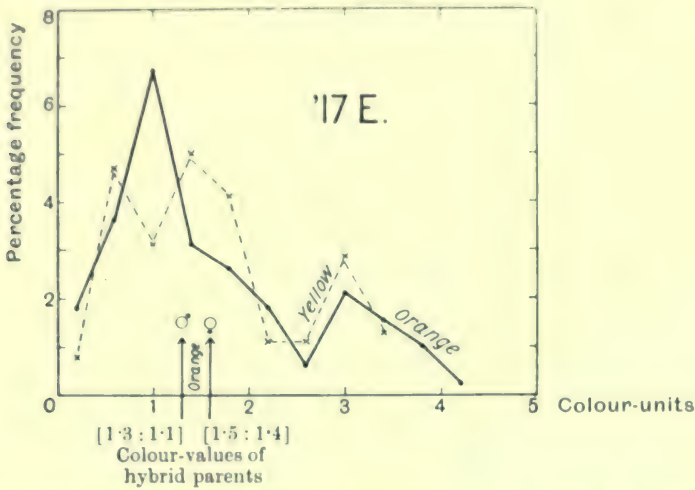


Fig. 11. (Cf. Fig. 23.) Curve showing frequency distribution of the orange and yellow colour-values of family '17 E, hybrid (*lutea* \times *gross.*) \times hybrid (*lutea* \times *gross.*).

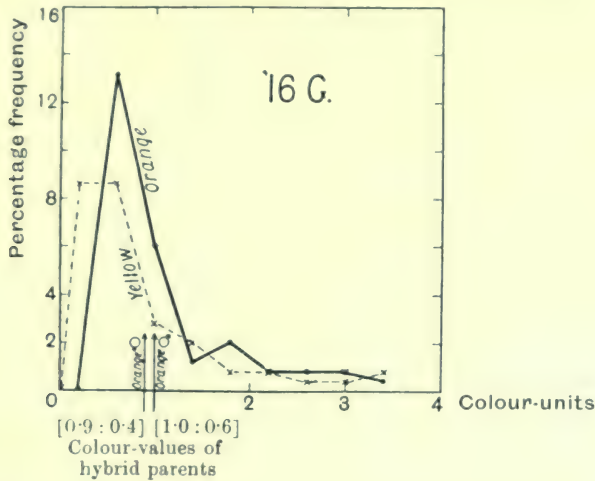


Fig. 12. (Cf. Fig. 24.) Curve showing frequency distribution of the orange and yellow colour-values of family '16 G, hybrid (*lutea* \times *gross.*) \times hybrid (*lutea* \times *gross.*).

The essential features are the same as in Fig. 10, but the curve, especially in the yellow region, tends to oscillate more. This is because the points on the curve have been taken rather closer together than

usual. The ratio of the area of the yellow class to the other two is about 22 : 78 (per cent.).

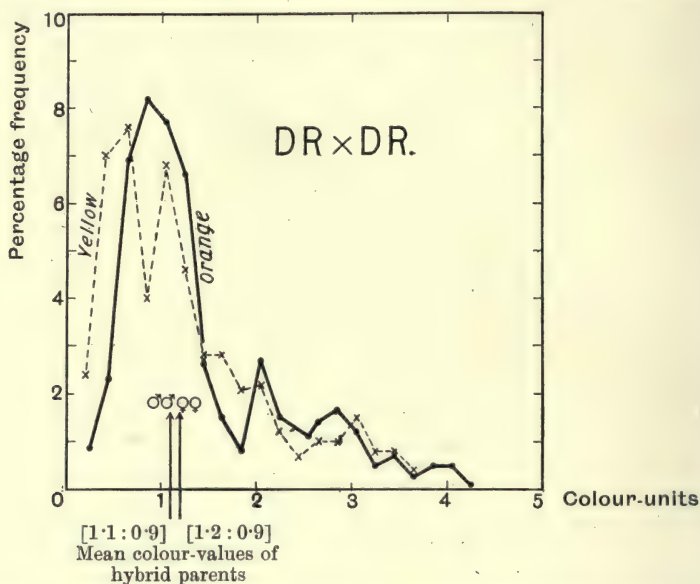


Fig. 13. Curve showing frequency distribution of the orange and yellow colour-values of the offspring of all the hybrid (*lutea* \times *gross.*) \times hybrid (*lutea* \times *gross.*) families shown in Figs. 10, 11, and 12.

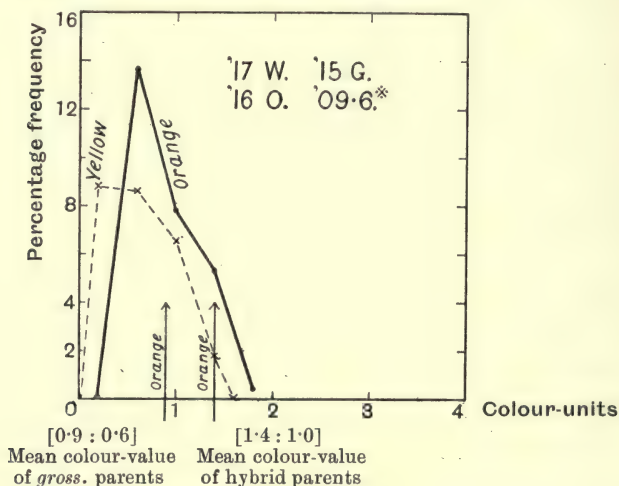


Fig. 14. (Cf. Fig. 25.) Curve showing frequency distribution of the orange and yellow colour-values of the offspring from four families of hybrid (*lutea* \times *gross.*) \times *A. grossulariata*.

(c) *Hybrid (lutea × gross.) × A. grossulariata.*

A few pairings only of this type of mating were made, and the four families resulting are shown in Fig. 14. With the exception of family '17 W the hybrid parents were all a very pale yellow, and the result, as might be expected, is a typical frequency distribution. The hybrid parent of '17 W had a high colour-value [2.4:2.2]. The offspring are much more numerous than is represented in Fig. 25. There were indeed 82 insects, but 64, which had a colour-value between [0.7:0.5] and [0.6:0.4] and which showed no other variations of interest, were set free as soon as they emerged. The insects of family '17 W which were entered in the curve (Fig. 25) may be found by reference to Table 1. The rather deep colour of the ♀ parent does not seem to have had any visible effect on the colour of the offspring.

VIII. SUMMARY.

The yellow ground colour of *Abraaxas grossulariata* var. *lutea* (Cockerell) is incompletely dominant over the white ground of the type. It was found impossible to divide the F_1 and F_2 generations into distinct classes, because the colours of the insects form a continuous series varying from white, through the palest yellows, to a deep orange.

A commercial instrument for the measurement of colours on an arbitrary standard scale of colour-units, called the "Tintometer," is described. By its means the colours of the insects have been given numerical values, thus enabling the complete data of the breeding experiments to be expressed in the form of curves showing the distribution of the colour-values. When these curves are converted into percentage frequency distributions, the F_1 generation resembles an ordinary chance distribution. But on the other hand the F_2 generation, etc. are at once seen to give curves having more than one maximum caused by the tendency of the colour factors to segregate according to the ordinary Mendelian laws.

Only some of the *DR* individuals are distinguishable from the *DD*, on account of a faint tinge of yellow in their wings. The difference between the two classes, when expressed in colour-units, is not sufficient to enable the maxima representing them to be separated on the curves.

The effects of sex and of variations of colour in different parts of the same insect upon the measurements are considered, and the various sources of experimental and statistical error are discussed. The gap on

the curves between the two classes is so clearly defined, and occurs so often in the same position in various families, that there can be no doubt it is significant and proves that even in the *DR* × *RR* families segregation really takes place.

A certain number of variations, which appeared during the course of the experiments, are described and figured. Very little is at present known of their genetics, but a table is given, enabling all details of their occurrence in the curves showing the distribution of the colour-values to be easily found. Further experiments are being conducted with var. *varleyata* as well as certain other varieties.

Special attention has been paid to the manner in which the pigments are deposited within the scale, and to the exact modifications which are the cause of certain varieties. Variations in the position and concentration of the melanic pigment are, for instance, the only factors which govern the difference between the two suffused varieties *iochalca* and *nigrosarsata*.

A microscopical examination of the scales, both *in situ* and when sectioned, shows that in the deepest colours the yellow pigment is diffused throughout the chitinous walls of the scales, without the formation of any granules. The intensity of the colour must therefore be determined, either by variations in the concentration, or by a change in the nature and consequently in the colour of the pigment itself. If the yellow variations are simply due to differences in the concentration of the colouring matter, suitable factors capable of controlling such quantitative changes must be formulated.

The chemical nature of the white and yellow pigments has been left for the subject of a further investigation.

[*Note added on July 19th, 1919.*] Since the above was written, the 1918 broods have emerged. On the whole the additional evidence from these insects (over 700) confirms the previous conclusions, but one fresh point of interest has been observed. In all the families previous to 1918 (except Dr Doncaster's) no *lacticolor* was used for pairing, lest this factor should complicate the case. In 1918 however the following pairings were made:

'18 V	<i>lacticolor</i>	♀	[0·8 : 0·8]	×	<i>lutea</i>	♂	[3·2 : 2·5]
'18 III	<i>lutea</i>	♀	[3·7 : 4·4]	×	<i>lacticolor</i>	♂	[0·9 : 0·8]
'18 V	<i>lutea</i>	♀	[3·2 : 3·5]	×	<i>lacticolor</i>	♂	[0·8 : 0·8]

In families '18 III and '18 V, the females were all *lacticolor*, since the ♂ parent was of this variety, and it was noticed that all these ♀ ♀ were

peculiarly pale, whereas most of the ♂♂ had an appreciable quantity of yellow. In family '18 V, for instance, there were 37 ♀♀ and 20 ♂♂, their respective colours being as follows:

Average colour of ♀♀ was		[1.1 : 1.0]
Average colour of ♂♂ was		[1.7 : 1.5]
5 ♀♀ were deeper than		[1.2 : 1.3]
10 ♂♂ " " "		[2.0 : 1.6].

Similarly, in the small family '18 III, there were 5 ♀♀ and 8 ♂♂, the respective colours being as follows:

Average colour of ♀♀ was		[1.1 : 1.1]
" " ♂♂ was		[2.1 : 1.9].

It was at first thought that there was some peculiarity in *lacticolor* which inhibits the development of the pale yellow colour characterising the heterozygous form of *grossulariata*. This seemed the more probable because one of Dr Doncaster's brood, already recorded, namely, family

'08.5 *lacticolor* ♀ [1.0 : 1.0] × *lacticolor* ♂ [4.0 : 3.0] (see Fig. 15),

contained, in spite of the deep colour of the ♂ parent, 11 ♂♂ and 14 ♀♀ only two of which were deeper than [1.2 : 1.4]. The remainder had an average colour-value of [1.0 : 1.0], there being practically no difference in the colour of the ♂♂ and the ♀♀.

In family '18 V, the ♀ parent was *lacticolor* and consequently both ♂♂ and ♀♀ were heterozygous *grossulariata*. A comparison between these ♀♀ and the *lacticolor* ♀♀ of the reciprocal cross shows that it is femaleness, or some factor associated with it, rather than *lacticolor* that prevents the development of the pigment in the pale yellow F_1 ♀♀. This can be seen from the fact that the *grossulariata* ♀♀ were even paler than the *lacticolor* ♀♀ from the reciprocal pairing, whereas the ♂♂ in the two families were approximately the same depth of yellow.

In family '18 V there were 23 ♀♀ and 23 ♂♂ the colours of which were as follows:

Average colour of ♀♀ was		[1.0 : 1.0]
Average colour of ♂♂ was		[1.4 : 1.6].

It was previously suggested on p. 219 that there was "a slight tendency for the ♀♀ to be paler than the ♂♂," but this difference was not more than 8—9% in the heterozygous ♀♀ either of the F_1 or F_2 generation. Although this point has not been tested (except in family '08.5) by

pairing a white to a yellow *laticolor*, the above explanation seems probable. The question is however doubtful and the experiments are being continued with a view to elucidating the problem.

I am indebted to Mr Raynor and Dr Doncaster not only for the material they have given me but for much kind help throughout the experiments and in preparing the paper. To Prof Punnett I am also grateful for encouragement and advice throughout the experiments. Mr Udny Yule has been kind enough to give me the benefit of his advice and opinion on the statistical points of the paper. I am especially indebted to Miss Moodie for her constant care of the larvae, etc. during several years, for without her cooperation the experiments would not have been possible.

TABLE I.

Table to enable individuals of any one family to be found in curves composed of more than one family.

Name of family	YELLOW TYPE (see Fig. 15)	Name of family	YELLOW YELLOW continued (see Fig. 16)
'17 B	♂ ♂ 8, 14, 19, 78, 79 ♀ ♀ 17, 22, 47, 49, 71, 81, 82	'17 β	♂ ♂ 22, 23 ♀ ♀ 1, 6, 7, 9, 10, 13, 14, 17, 48
'17 K	♂ ♂ 11, 18, 33, 39, 51, 72, 83 ♀ ♀ 12, 24, 34, 57, 127	'17 δ	♂ ♂ 39, 52, 53 ♀ ♀ 19, 41, 64
'17 a	♂ ♂ 1, 2, 5, 7, 25 ♀ ♀ 20, 26, 31, 121	'17 ε	♂ ♂ 2
'17 e	♂ ♂ 15 ♀ ♀ 65, 67, 111, 112, 114	'17 η	♂ ♂ 51 ♀ ♀ 25, 93
'16 P	♂ ♂ 4, 6, 10, 21, 48, 52, 117, 129 ♀ ♀ 3, 80, 131, 136	'17 θ	♂ ♂ 49, 54 ♀ ♀ 4, 15, 18, 38
'16 T	♂ ♂ 35, 44, 119, 125 ♀ ♀ 36, 59, 84, 107, 122, 126, 128	'17 μ	♂ ♂ 27
'15 C	♂ ♂ 32, 62, 146, 148, 149, 150 ♀ ♀ 55, 115, 123, 140	'16 B	♂ ♂ 16, 21, 28, 33, 40, 44, 50, 63, 65, 66, 69, 73, 80, 82, 92, 95, 98 ♀ ♀ 42, 61, 74, 85, 86, 91, 99, 100
'15 F	♂ ♂ 56	16 D	♀ ♀ 96
'15 K	♂ ♂ 40, 113, 133, 141, 144 ♀ ♀ 53, 145	HYBRID - YELLOW (see Fig. 17)	
'15 M	♂ ♂ 13, 29, 37, 38, 41, 42, 43, 50, 54, 68, 69, 73, 77, 108, 109, 120, 124 ♀ ♀ 132, 137	'17 O	♂ ♂ 21, 33, 66, 76, 79, 80, 87, 88, 135 ♀ ♀ 8, 28, 29, 119
'15 Q	♂ ♂ 85, 135 ♀ ♀ 16, 75, 110, 130, 138	'17 R	♂ ♂ 16, 18, 22, 27, 30, 41, 71, 72, 81, 112, 113, 116, 132, 139, 140 ♀ ♀ 47, 59, 73, 82, 94, 97, 100, 101, 102, 106, 118, 121, 128, 129, 130, 131
'14 B	♂ ♂ 9, 23, 46, 76, 86, 104, 106, 116, 118 ♀ ♀ 30, 45, 105	'17 Y	♂ ♂ 14, 24, 49, 77, 99, 124, 138 ♀ ♀ 11, 12, 19, 20, 103, 110, 114, 136
'08-5*	♂ ♂ 27, 63, 64, 66, 87, 88, 89, 90, 91, 92, 93 ♀ ♀ 28, 58, 60, 61, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103	'16 H	♂ ♂ 3, 7, 45, 83, 90, 111 ♀ ♀ 2, 34, 65, 68, 120, 122, 142
'08-20*	♂ ♂ 74, 143, 147, 151 ♀ ♀ 70, 134, 139, 142	'15 E	♂ ♂ 10, 31, 32, 38, 40, 43, 44, 50, 58, 74 ♀ ♀ 1, 6, 13, 15, 37, 78, 107
YELLOW - YELLOW (see Fig. 16)		'15 N	♂ ♂ 46, 53, 60 ♀ ♀ 25, 35, 51, 52, 89, 92, 123
'17 D	♂ ♂ 29, 30, 37, 59, 60, 67, 76, 77, 78, 79, 90, 94 ♀ ♀ 24, 26, 88, 97	'15 O	♂ ♂ 5, 23, 95 ♀ ♀ 75, 91
'17 F	♂ ♂ 45, 58 ♀ ♀ 75, 87	'15 P	♂ ♂ 4, 48, 57, 69, 84, 85, 86, 93, 104, 108, 125 ♀ ♀ 9, 64, 141
'17 H	♂ ♂ 46, 47 ♀ ♀ 20, 32, 36	'15 R	♀ ♀ 96, 133
'17 S	♂ ♂ 43	'14 A	♂ ♂ 17, 26, 39, 42, 54, 63, 70, 105, 115, 134 ♀ ♀ 36, 55, 56, 61, 62, 67, 98, 109, 117, 126, 127, 137
'17 T	♂ ♂ 71, 81, 83, 84, 89 ♀ ♀ 3, 5, 8, 11, 12, 31, 34, 35, 55, 56, 57, 62, 68, 70, 72		

* Bred by Dr Doncaster.

TABLE I—continued.

Name of family	HYBRID×HYBRID (see Fig. 22)	Name of family	HYBRID×HYBRID—continued (see Fig. 22)
'17 P	♂ ♂ 20, 22, 52, 67 ♀ ♀ 128	'15 S	♂ ♂ 28, 86, 117, 118 ♀ ♀ 15, 19, 29, 43, 85, 124
'17 Q	♂ ♂ 8, 23, 24, 74, 75, 80, 83, 112, 175, 176 ♀ ♀ 18, 73, 114	'07-23*	♂ ♂ 2, 7, 10, 11, 14, 51, 59, 65, 94, 95, 96, 101, 103, 119, 120, 182 ♀ ♀ 1, 3, 8, 31, 32, 40, 48, 53, 55, 58, 61, 63, 77, 87, 88, 89, 97, 104
'17 UU	♂ ♂ 76, 148, 149, 164, 167, 177 ♀ ♀ 6, 108, 147, 165, 166	'09-19*	♂ ♂ 35, 69, 78, 131, 152, 191, 192, 195, 196 ♀ ♀ 4, 34
'16 C	♂ ♂ 36, 41, 79, 84, 116, 189, 190 ♀ ♀ 188, 197, 199, 200	'10-12*	♂ ♂ 12, 54, 57, 66, 98, 102, 105, 111, 121, 122, 123, 135, 136, 146, 163 ♀ ♀ 5, 44, 62, 90, 91, 92, 132
'16 S	♂ ♂ 183, 184, 187, 198		
'16 KF	♂ 100		
'16 M	♂ ♂ 30, 60, 81, 168, 169, 186 ♀ ♀ 115, 129, 144, 145, 153		
'16 N	♂ ♂ 25, 37, 39, 46, 47, 68, 106, 126, 138, 142, 154, 162, 171, 178, 194 ♀ ♀ 70, 71, 72, 82, 107, 125, 127, 134, 139, 140, 141, 157, 158, 159, 160, 170, 172, 179, 180, 181, 193		
			HYBRID×TYPE (see Fig. 25)
'16 R	♂ ♂ 33, 45, 50, 56, 161 ♀ ♀ 113, 143, 155, 173, 185	'17 W	♂ ♂ 28, 38, 39, 40, 41, 42, 43, 57, 58 ♀ ♀ 16, 23, 24, 25, 33, 34, 35, 36, 44
'16 V	♂ ♂ 49, 137, 156, 174 ♀ ♀ 26, 27, 42, 99, 133	'16 O	♂ 37 ♀ ♀ 21, 32
'15 D	♂ ♂ 109, 130, 151 ♀ ♀ 150	'15 G	♂ ♂ 22, 26 ♀ 31
'15 L	♂ ♂ 13, 16, 17, 38, 64, 110 ♀ ♀ 21, 93	09-6*	♂ ♂ 1, 2, 3, 4, 5, 6, 9, 17, 27, 29, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56 ♀ ♀ 7, 8, 10, 11, 12, 13, 14, 15, 18, 19, 20, 30

N.B. All the above numbers denote positions of insects along the base lines of the following distribution figures.

* Bred by Dr Doncaster.

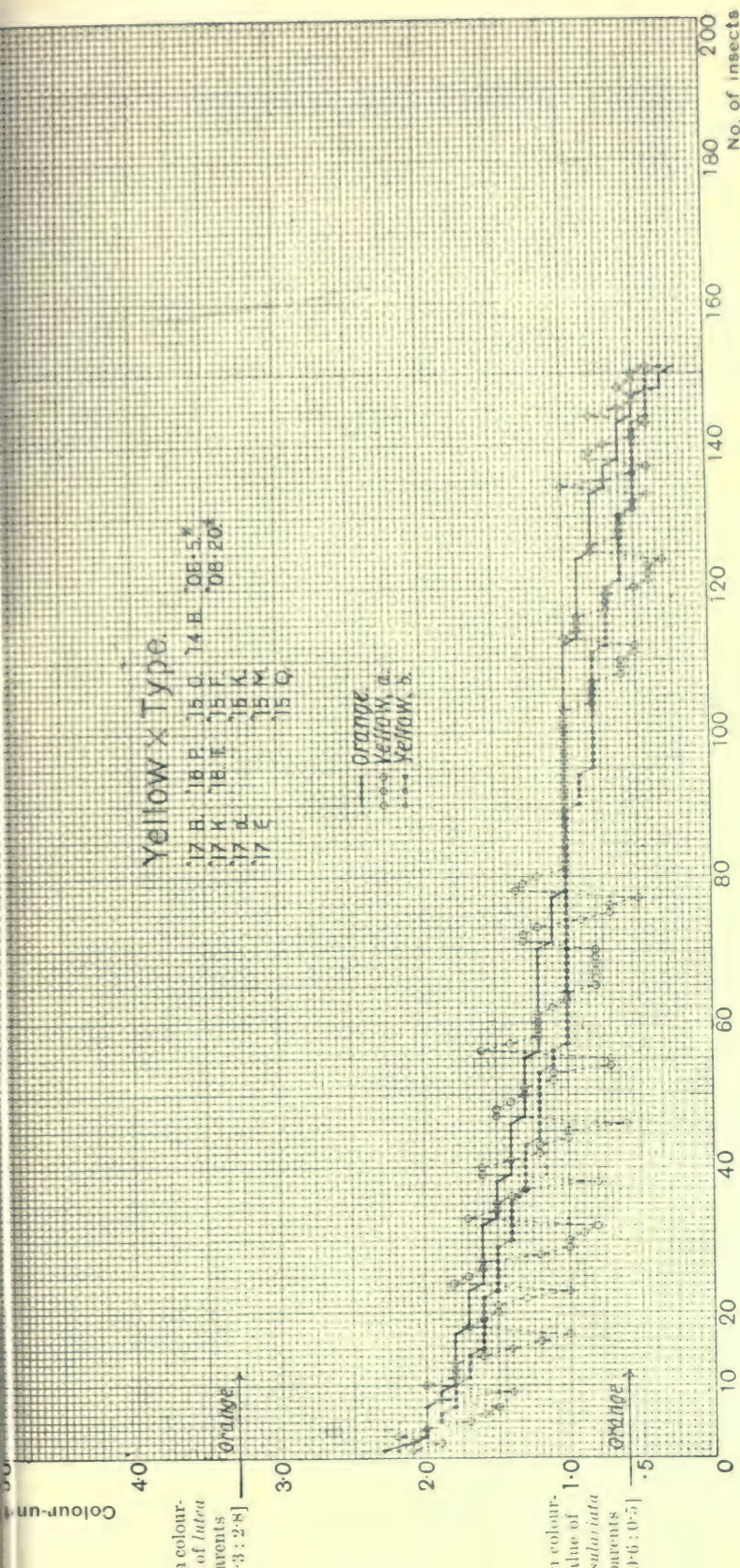


Fig. 15. (Cf. Fig. 2.) Curve showing the distribution of the orange and yellow colour-values of the offspring from 14 pairings of *lutea* and *granulata*. The following variations occur in certain insects, whose position along the base line of the curve is indicated by the accompanying numbers.

Family '17 B. <i>nigroparsata</i> 579	Family '17 K. <i>nigrocineta</i> + 539 <i>nigrocineta</i> + 34 <i>nigrocineta</i> ++ 34 <i>nigrocineta</i> ++ 83 (left wing - right wing) <i>nigrocineta</i> ++ 127 <i>nigrocineta</i>	Family '16 T. <i>nigrocineta</i> + 119 <i>hagelshensis</i> 128	Family '15 K. <i>nigrocineta</i> + 133
Family 08.5.3. ♂ 27, 63, 64, 66, 87, 88, 89, 90, 91, 92, 93 ♀ 28, 58, 60, 61, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103 <i>Lactidior</i>	Family 17.7. ♀ 94, 95, 96, 97 <i>Padrapurata</i>		

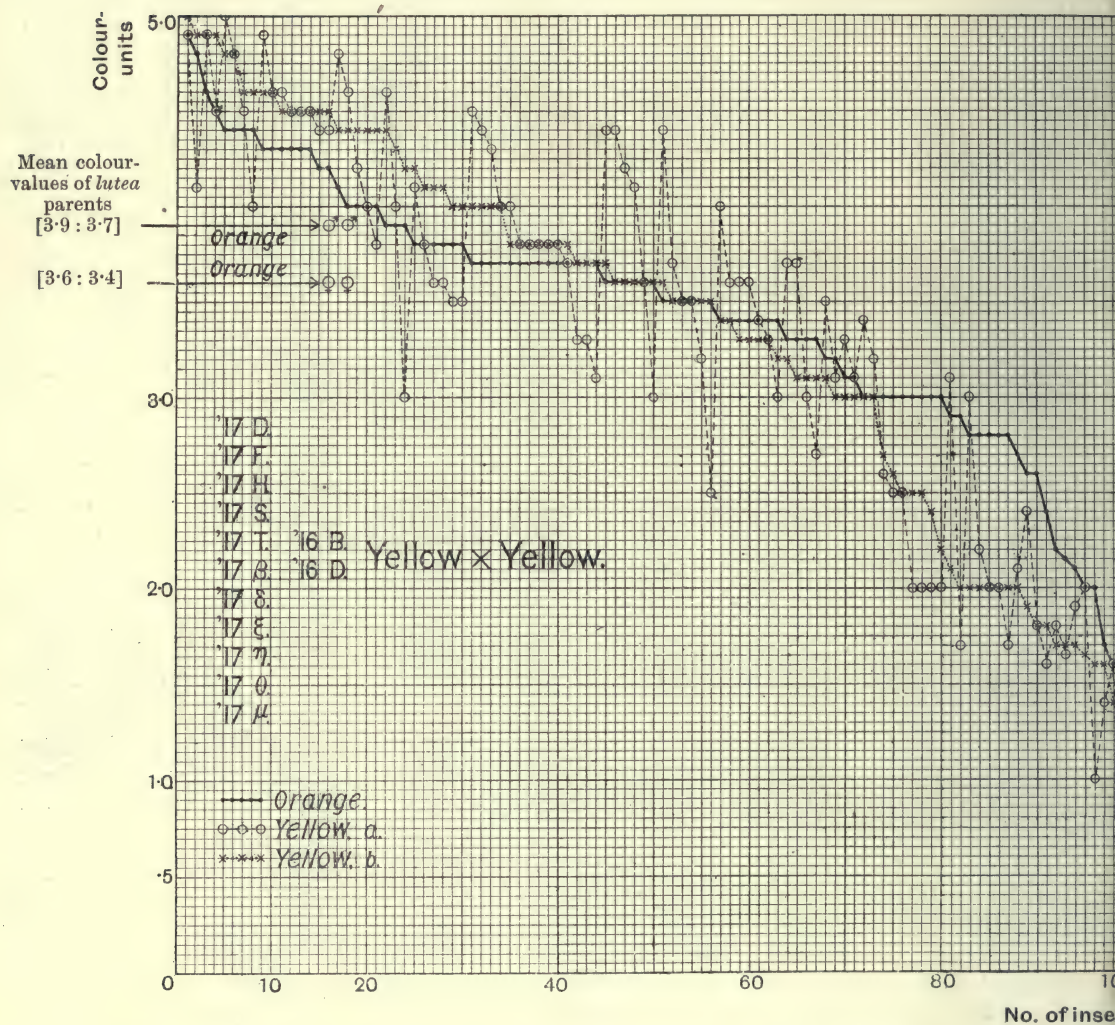


Fig. 16. (Cf. Fig. 3.) Curve showing the distribution of the orange and yellow colour-values of the offspring from 13 pairings of *lutea* × *lutea*.

These families included no variations.

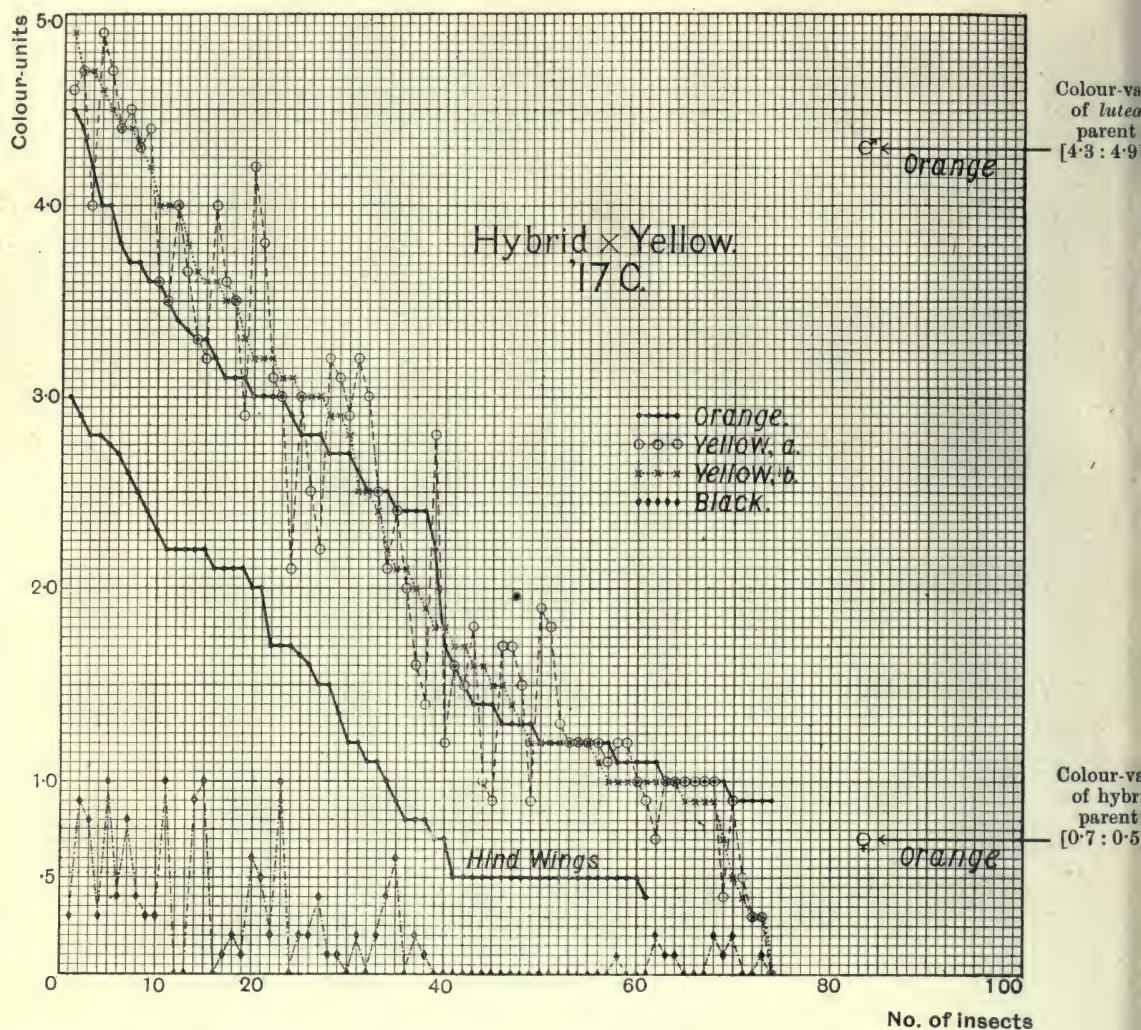


Fig. 18. (Cf. Fig. 5.) Curve showing the distribution of the orange and yellow colour-values of family '17 C, hybrid (*lutea* \times *gross.*) \times *lutea*.

Two separate curves are added showing the black values of the fore wings, and the orange values of the hind wings. The following variations occur in certain insects, whose position along the base line of the curve is indicated by the accompanying numbers.

- nigricostata* + ♂ 36, 37, 38, 45, 49, 51, 52, 53 (radiated), 58,
61 (radiated), 62, 70
 ,, ,, ♀ 3, 14, 30, 63 (radiated), 74 (*nigrocincta* +)
 ,, ++ ♂ 50, 69
nigrocincta ++ ♀ 35 (slightly radiated)

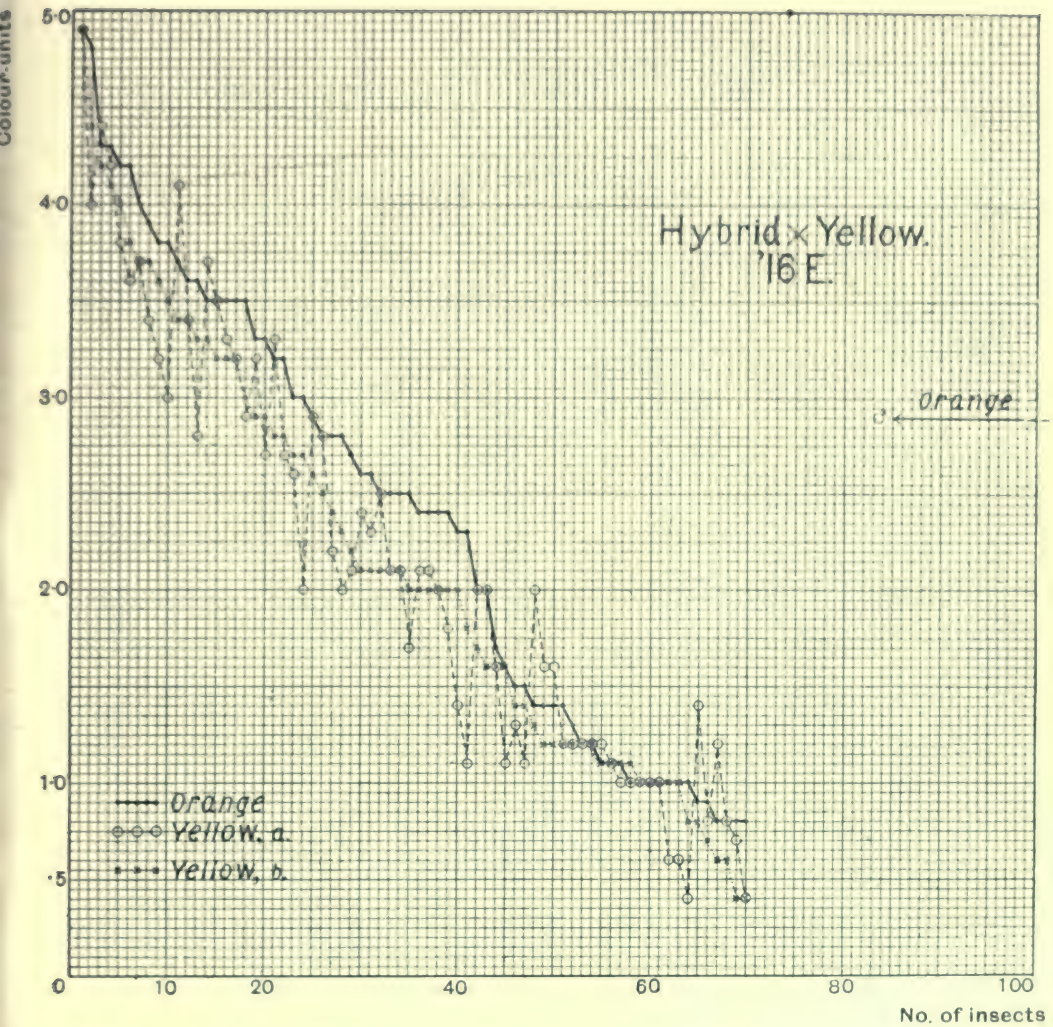


Fig. 19. (Cf. Fig. 6.) Curve showing the distribution of the orange and yellow colour-values of family '16 E, hybrid (*lutea* × *gross.*) × *lutea*.

The following variations occur in certain insects, whose position along the base line of the curve is indicated by the accompanying numbers.

Very broad orange fascia ♀ 35.

nigricostata + ♂ 32. : : 66, 70

.. ++ ♂ 61

nigrocincta + ♀ 2, 23 (segments 1, 2 and 3)

.. ++ ♀ 29

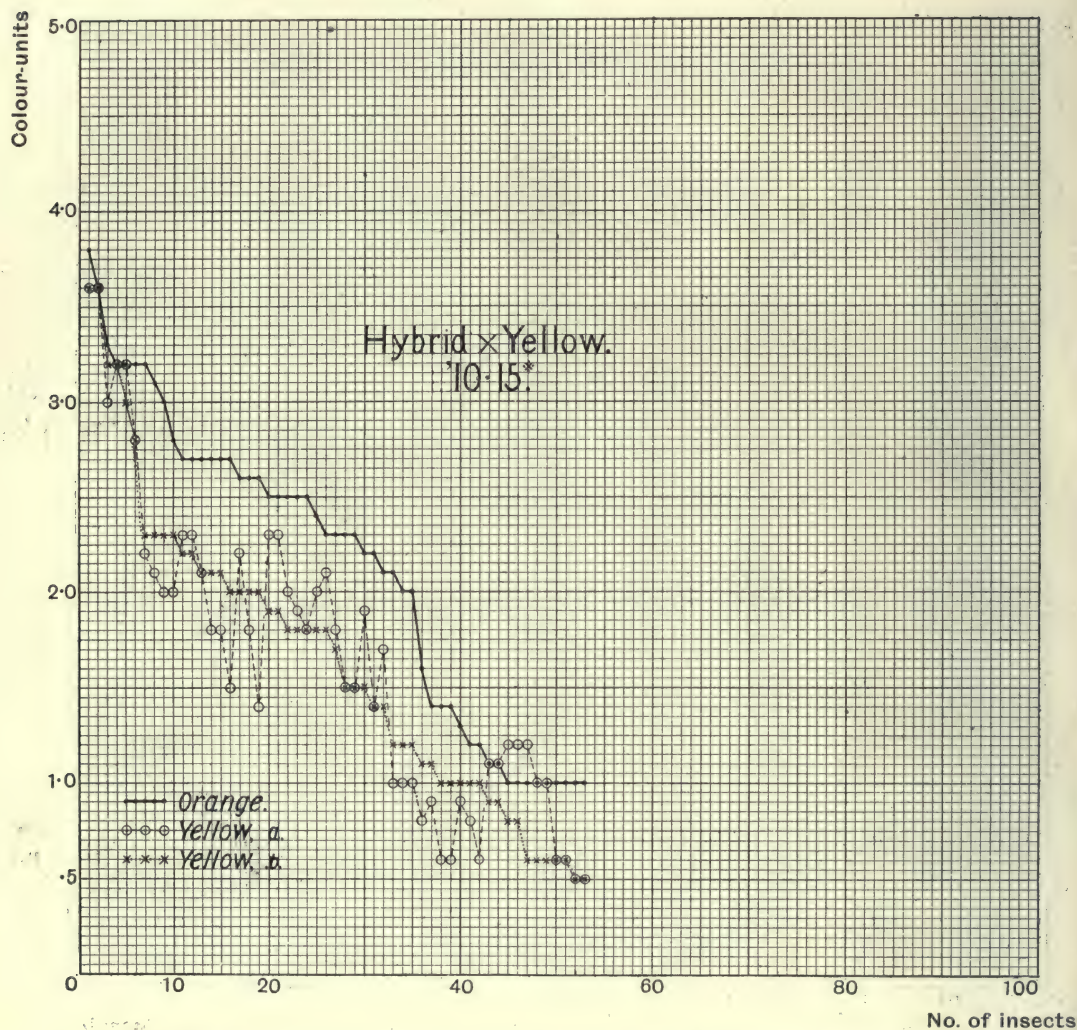


Fig. 20. (Cf. Fig. 7.) Curve showing the distribution of the orange and yellow colour-values of family '10-15*', hybrid (*lutea* x *gross.*) x *lutea*. Parents missing.

The following variations occur in certain insects, whose position along the base line of the curve is indicated by the accompanying numbers.

<i>lacticolor</i>	♂ 2, 4, 5, 14, 20, 23, 24, 25, 27, 28, 30,	<i>nigricostata</i> ♂ 19, 20, 23, 34, 36
	31, 33, 34, 35, 36, 38, 39	„ + ♀ 1, 10, 15, 47
	♀ 3, 6, 18, 26, 29, 42, 50, 51, 52, 53	„ ++ ♂ 13, 37, 41
<i>fulvaticata</i>	♂ 9, 11, 19, 20, 23, 27, 28, 39, 46, 48, 49	„ ## ♂ 48, ♀ 45
	♀ 1, 6, 10, 14, 17, 18, 36, 43, 45, 51	<i>semiviolacea</i> ♂ 9
		<i>A. gross.</i> (type) ♂ 12, ♀ 8, 22

* Bred by Dr Doncaster.

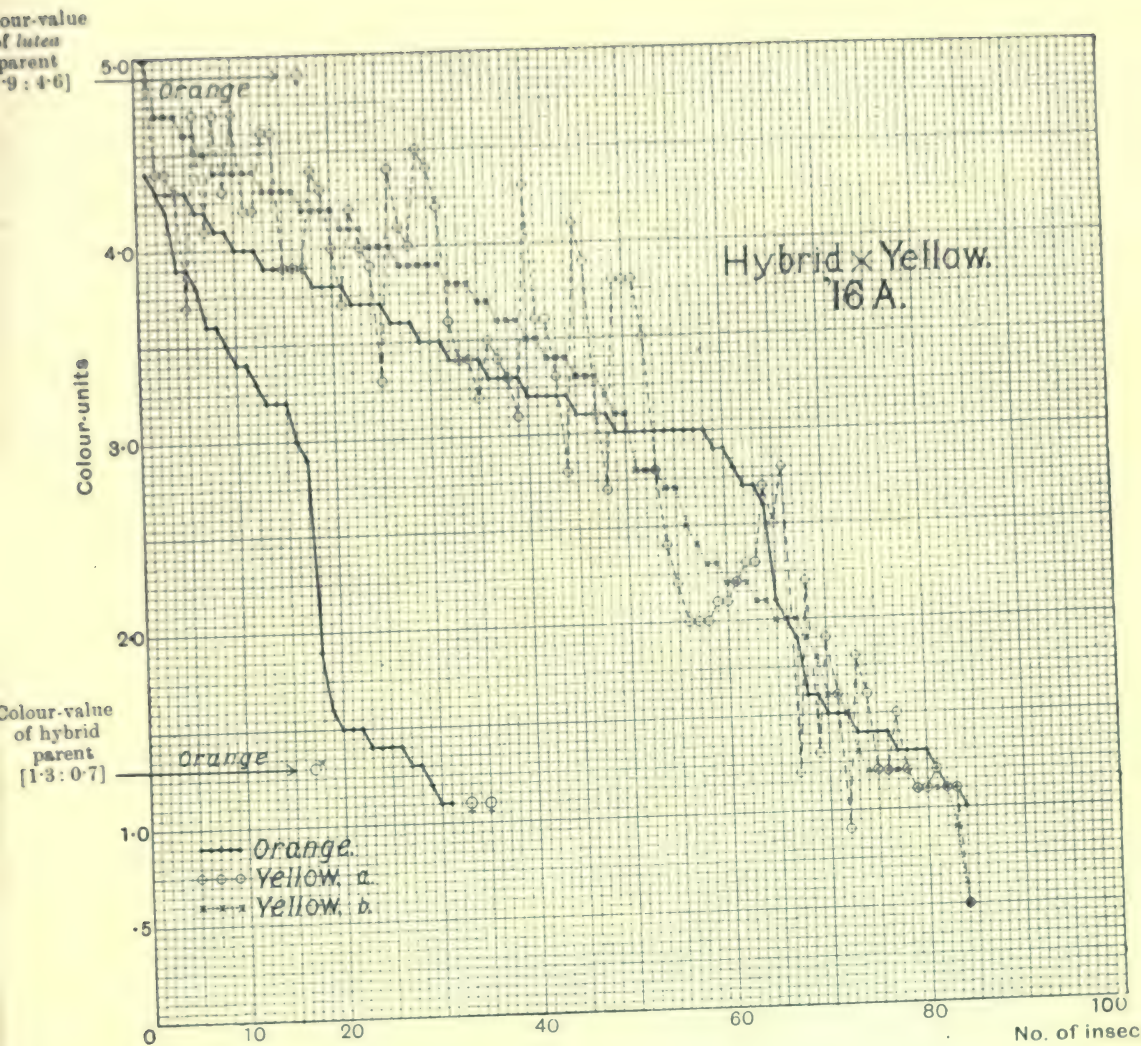


Fig. 21. (Cf. Fig. 8.) Curve showing the distribution of the orange and yellow colour-values of family '16 A, hybrid (*lutea* x *gross.*) x *lutea*.

The following variations occur in three insects, whose position along the base line of the curve is indicated by the accompanying numbers.

nigricostata 73 *nigrocincta* 70 (segments 2 and 3). Broad orange fascia 69, 72
nigrocincta

Hybrid x Hybrid.

17P. 16C. 15D. 07.23*
 17Q. 16M. 15L. 06.18*
 17U. 16N. 15S. 10.12*
 16R. 16S.
 16V. 16E.

Orange
 Yellow, a.
 Yellow, b.

Mean colour-
 values of
 hybrid
 parents
 [1:1:0:9]
 [1:0:0:9]

Orange
 Yellow, a.
 Yellow, b.

No. of insects

Fig. 22. (Cf. Fig. 10.) Curve showing the distribution of the orange and yellow colour-values of the offspring from 16 pairings of hybrid (*tutea* x *gross.*) x *tutea*.

The following variations occur in certain insects, whose position along the base line of the curve is indicated by the accompanying numbers.

Family '17 P.

nigricostata + 567

Family '16 N.

impunctifasciata + 72

++ ♀ ♀ 71 (violacea), 139, 180, 181

impunctifasciata ++

nigricostata +

nigricostata ++ 170

+ 37

♀ 70, 82 (violacea), 159,

172, 179

Family '17 Q.

impunctifasciata + 175

++ 174

++ 75

Family '16 R.

semiviolacea ♀ 143 (fore wings only)

Family '07-23*.

lacticolor ♂ 2, 14, 51, 59, 103, 119

♀ 1, 3, 8, 40, 48, 53, 55, 58, 63, 87, 88, 89

nigricostata + 395, ♀ 104

semiviolacea (hind wings only) ♂ 101

Family '17 UU.

impunctifasciata + 164

++ 166

++ ♀ 147, 165

Family '16 V.

nigricostata + 174

Family '10-12*

lacticolor ♂ 98, 102, 105, 121, 122, 123, 146

♀ 44, 90, 91, 92

Family '16 M.

impunctifasciata +

nigricostata

impunctifasciata ++ 115

Family '09-19*.

lacticolor ♀ 4, 34

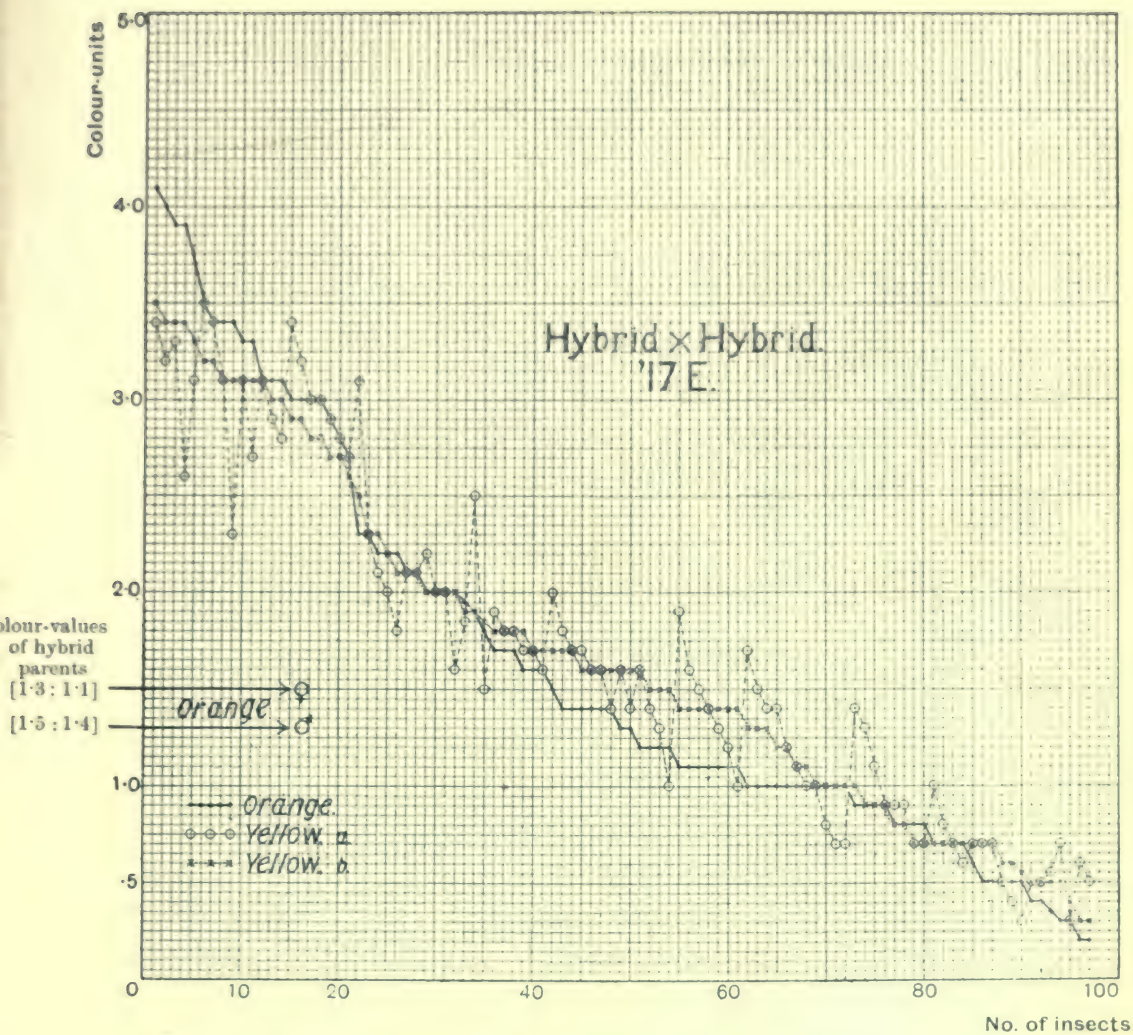


Fig. 23. (Cf. Fig. 11.) Curve showing the distribution of the orange and yellow colour-values of family '17 E, hybrid (*lutea* × *gross.*) × hybrid (*lutea* × *gross.*).

The following variations occur in certain insects, whose position along the base line of the curve is indicated by the accompanying numbers.

nigrocincta (segments 3, 4 and 5) ♀ 60

nigrocincta ♂♂ 4, 93, 97

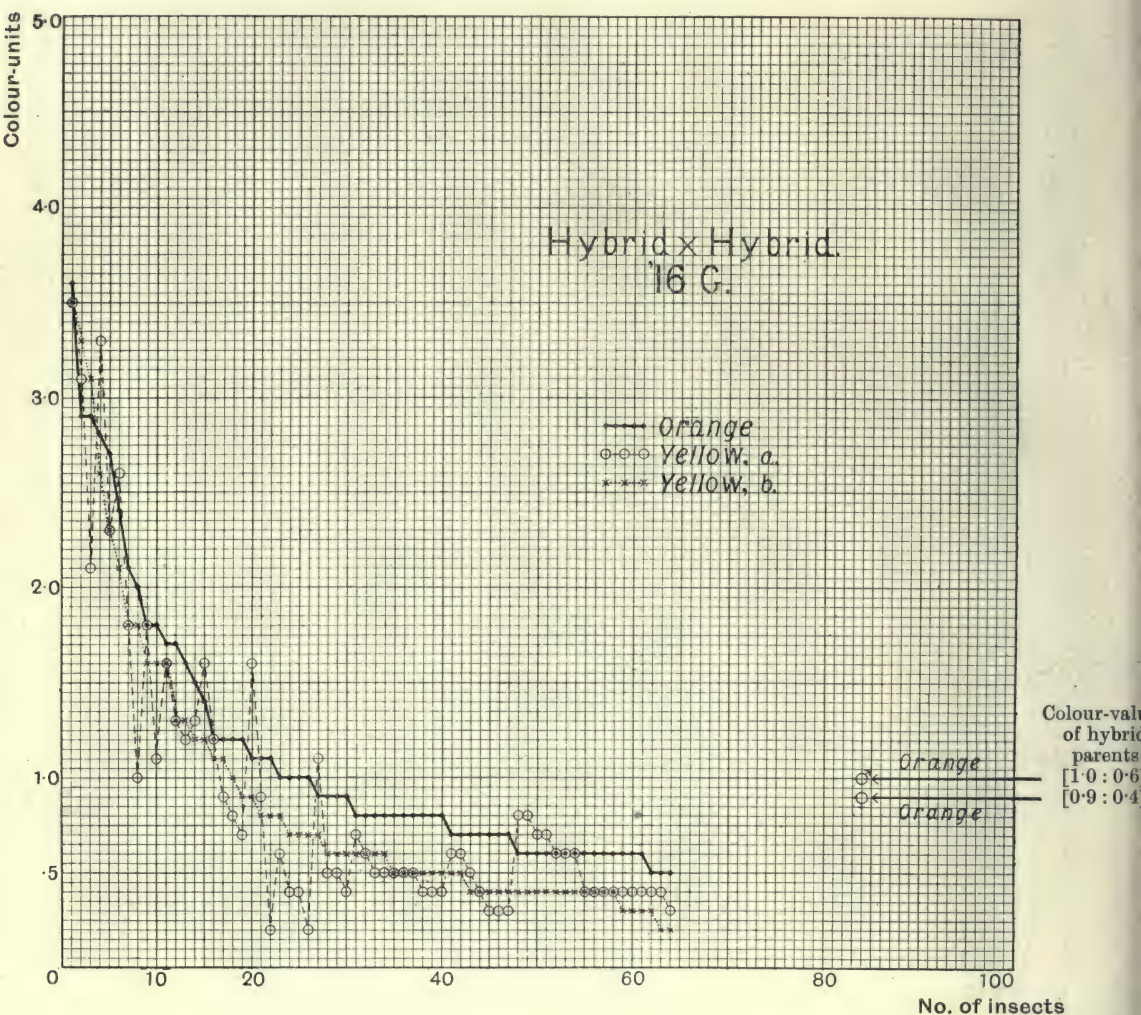
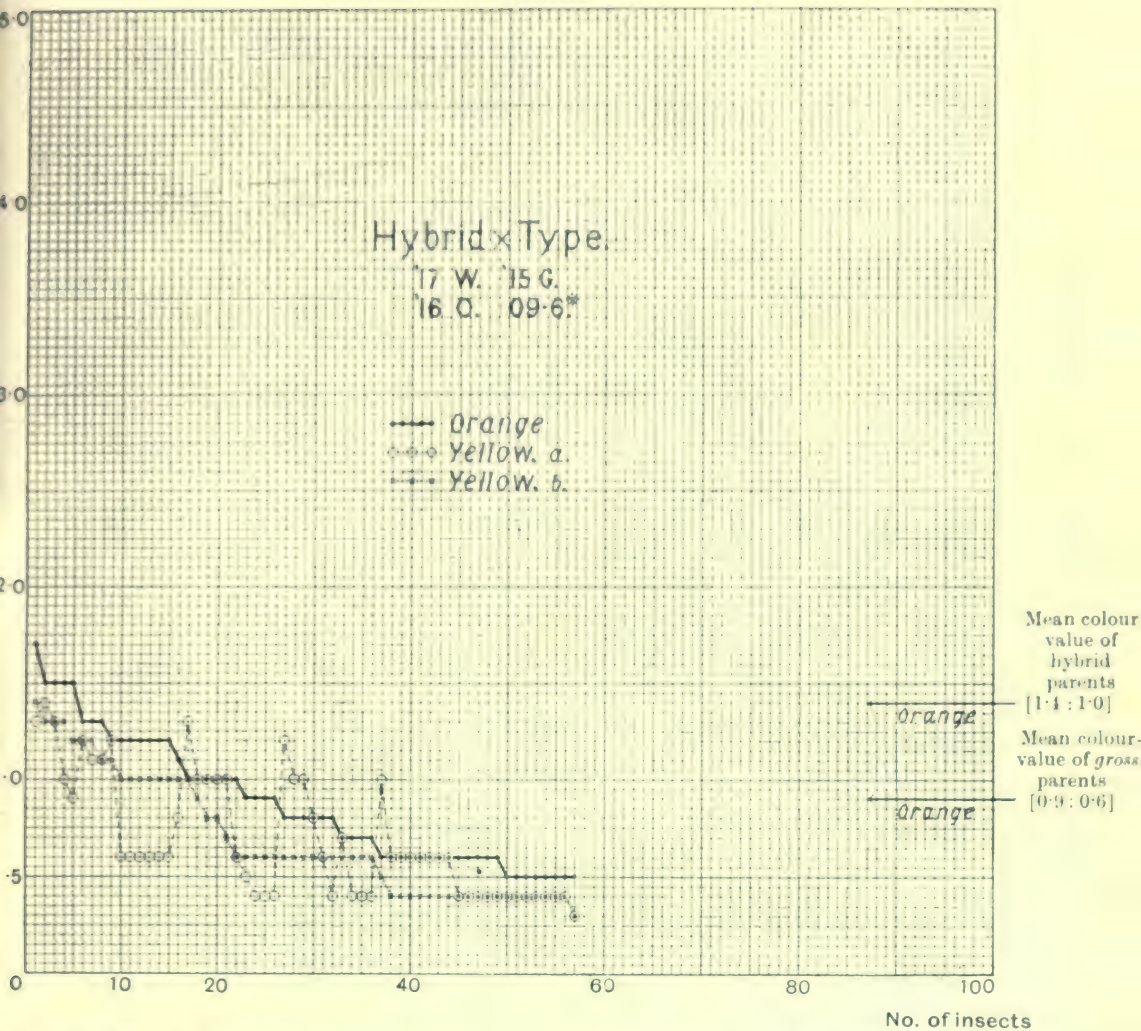


Fig. 24. (Cf. Fig. 12.) Curve showing the distribution of the orange and yellow colour-values of family '16 G, hybrid (*lutea* x *gross.*) x hybrid (*lutea* x *gross.*).

The following variations occur in certain insects, whose position along the base line of the curve is indicated by the accompanying numbers.

<i>nigricostata</i> + ♂ 13, 15, 45, 61	<i>violacea</i> ♂ 24	<i>semiviolacea</i> } ♂ 27
„ „ (right wing only) ♂ 62	<i>semiviolacea</i> ♀ 7, 8 (fore wings only)	<i>nigricostata</i> + } ♂ 27
„ „ (left wing only) ♂ 47		



25. (Cf. Fig. 14.) Curve showing the distribution of the orange and yellow colour-values of the offspring from four pairings of hybrid (*lutea* × *gross.*) × *A. grossulariata*.

In addition to the 8 ♀♀, 9 ♂♂ included above, family 17 W contained 64 insects whose colour-value was less than [0.7 : 0.5].

The following variations occur in certain insects, whose position along the base line of the curve is indicated by the accompanying numbers.

Family 17 W.

impunctifasciata + ♂♂ 41, ♀♀ 16

“ ++ ♂♂ 28, ♀♀ 23, 24, 25, 44

ulvaticata ♀♀ 23

nigricostata ++ ♂♂ 28, 44, 58

“ “ ♀♀ 34, 35

hazleighensis ♂♂ 38, 39, 40, 41, 43

“ “ ♀♀ 33, 36

Family 09-6 ×.

nigricostata + ♂♂ 3, 9, 17, 27, 45, 46, 48, 50

lacticolor ♂♂ 1, 2, 3, 4, 5, 6, 9, 17, 27, 29, 45, 46, 47, 48, 49

50, 51, 52, 53, 54, 55, 56

“ ♀♀ 7, 8, 10, 11, 12, 13, 14, 15, 18, 19, 20, 30

* Bred by Dr Doncaster.

DESCRIPTION OF PLATES.

PLATE IX.

Abraaxas grossulariata.

Reduced two-thirds natural size.

1—12. Var. *lutea*.

1—6. Orange colour-values from 3·2—4·0 and over.

1. Var. *flavipalliata* ♀.
2. Var. *lutea* ♂.
3. Var. *fulvopicata* ♂.
4. Var. *lutea* ♀.
5. Var. *lutea* ♀.
6. Var. *lutea* ♂.

7—12. Orange colour-values from 3·2 to 2·4.

7. Var. *flavipalliata* ♂.
8. Var. *lutea* ♀.
9. Var. *flavipalliata* ♂.
10. Var. *lutea* ♂.
11. Var. *semiviolacea* ♀.
12. Var. *lutea* ♂.

13—24. Pale yellow hybrids (*lutea* × *A. grossulariata*).

13—18. Orange colour-values from 2·4 to 1·6.

13. Pale hybrid ♂.
- *14. Pale hybrid ♀.
- *15. Pale hybrid ♂.
- *16. Var. $\left\{ \begin{array}{l} \textit{nigrocincta} \text{ ++} \\ \textit{nigricostata} \text{ +++} \end{array} \right. \text{ ♀.}$
- *17. Pale hybrid ♂.
- *18. Pale hybrid ♀.

19—24. Orange colour-values from 1·6 to 0·8.

19. Var. *flavipalliata* ♀.
20. Pale hybrid ♂.
21. Var. $\left\{ \begin{array}{l} \textit{fulvopicata} \\ \textit{impunctifasciata} \text{ ++} \end{array} \right. \text{ ♀.}$
22. Var. $\left\{ \begin{array}{l} \textit{nigricostata} \text{ +} \\ \textit{semiviolacea} \end{array} \right. \text{ ♀.}$
23. Pale hybrid ♀.
24. Var. *impunctifasciata* ++ ♀.

* [Owing to an error in the reproduction which could not be corrected, insects Nos. 14, 15, 16, 17, and 18, especially the last, appear very much too dark in colour. They should be somewhat lighter than Nos. 12 and 13 and slightly darker than the insects in the next column, Nos. 19 and 20.]

25 and 26. *A. grossulariata*. Orange colour-values from 0.0 to 0.8.

25. Type ♀. (Bexley, Kent.)

26. Type ♂ bred by W. Newman.

27. Var. *semiviolacea* ♀.

28. Var. $\begin{cases} \textit{fulvapicata} \\ \textit{nigricostata} \\ \textit{semiviolacea} \end{cases}$ ♀.

29. Var. *nigrosparcata* ♂. (Bexley, Kent.)

30. Var. $\begin{cases} \textit{hazeleighensis} \\ \textit{nigrocincta} \end{cases}$ ♂.

31—60. Var. *lacticolor*.

31—42. Var. *chrysostrota*.

31—36. Orange colour-values from 3.2 to 4.0 and over.

31. Var. *chrysostrota* ♀.

32. Var. *chrysostrota* ♀.

33. Var. *chrysostrota* ♂.

34. Var. *chrysostrota* ♀.

35. Var. *chrysostrota* ♂.

36. Var. *chrysostrota* ♀.

37—42. Orange colour-values from 3.2 to 2.4.

37. Var. *chrysostrota* ♂.

38. Var. $\begin{cases} \textit{chrysostrota} \\ \textit{fulvapicata} \end{cases}$ ♀.

39. Var. $\begin{cases} \textit{chrysostrota} \\ \textit{fulvapicata} \end{cases}$ ♂.

40. Var. $\begin{cases} \textit{chrysostrota} \\ \textit{fulvapicata} \end{cases}$ ♀.

41. Var. *chrysostrota* ♀.

42. Var. *chrysostrota* ♂.

43—54. Pale yellow hybrids (*chrysostrota* × *lacticolor*).

43—48. Orange colour-values from 2.4 to 1.6.

43. Var. $\begin{cases} \textit{chrysostrota} \\ \textit{fulvapicata} \end{cases}$ ♀.

44. Var. $\begin{cases} \textit{chrysostrota} \\ \textit{fulvapicata} \\ \textit{nigricostata} \end{cases}$ ♀.

45. Var. $\begin{cases} \textit{chrysostrota} \\ \textit{fulvapicata} \end{cases}$ ♂.

46. Pale hybrid.

47. Var. $\begin{cases} \textit{chrysostrota} \\ \textit{fulvapicata} \end{cases}$ ♀.

48. Pale hybrid.

49—54. Orange colour-values from 1.6 to 0.8.

49. Pale hybrid ♀.

50. Pale hybrid ♀.

51. Pale hybrid ♂.

52. Pale hybrid ♀.

53. Pale hybrid ♀.

54. Pale hybrid ♀.

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55 and 56. Orange colour-values from 0.0 to 0.8.

55. Var. *lacticolor* ♀.

56. Var. *lacticolor* ♀.

57. Var. *lacticolor* ♀.

58. Var. *radiata* ♀.

59. Var. *cupreofasciata* ♀.

60. Var. *iochalca* ♂.

Nos. 1, 7, 59 and 60 were very kindly given to me by the Rev. G. H. Raynor, all the other specimens were bred during the course of the experiments.

PLATE X.

1 and 2 were sketched with a Zeiss AA objective and ocular 5 giving a magnification of about 200 diameters.

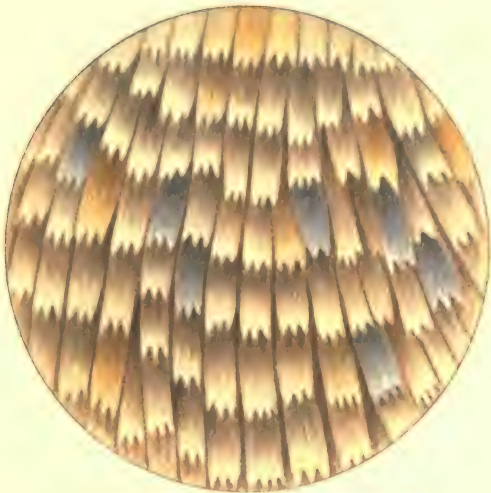
3, 4 and 5 were drawn with the camera and a Leitz 1/20 inch oil immersion objective and ocular 9, giving a magnification of about 1360 diameters.

1. Portion of fore wing of var. *iochalca* (see Plate IX, No. 60) showing the way in which the melanic pigment is diffused throughout the scales. The base of the scales contains most pigment but the effect is masked by the pale overlapping tips of those in the preceding row. There are a few grey scales, but not sufficient to modify the colour appreciably.
2. Portion of the fore wing (near the costa) of var. *nigrosparsata* with yellow, not white, ground (as in Plate IX, No. 29) showing the way the melanic pigment is concentrated in certain scales. The tips of the melanic scales usually contain more pigment than the base, and further the yellow scales are almost devoid of black, which gives the insect a faintly mottled appearance.
- 3, 4, and 5. Unstained sections of the scales, about 2 to 3 μ thick, of var. *lutea* from specimens of varying depths of colour, showing the way in which the yellow pigment is diffused throughout the chitin of the walls of the scale. Even in the deepest yellow insect (3) there is no granular pigment.



Orange values from 3.2 to 4.0 and over
Orange values from 2.4 to 3.2
Orange values from 1.6 to 2.4
Orange values from 0.8 to 1.6
Orange values from 0.0 to 0.8
Orange values of Nos. 25 and 26 0.0 to 0.8





1



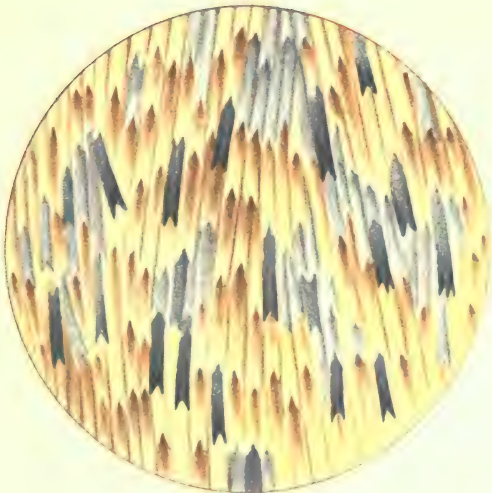
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4



5



2

STUDIES IN THE HYBRID BISTONINAE.

III. THE STIMULUS OF HETEROZYGOSIS.

By J. W. HESLOP HARRISON, D.Sc.

(With Two Text-figures.)

PARTLY owing to the influence of Darwin and those who succeeded him, and partly because it harmonises with the observed facts, the dictum that cross fertilisation is a source of strength or of stimulus to metabolic activity has become almost axiomatic; very few workers, however, seem to have speculated as to its method of action.

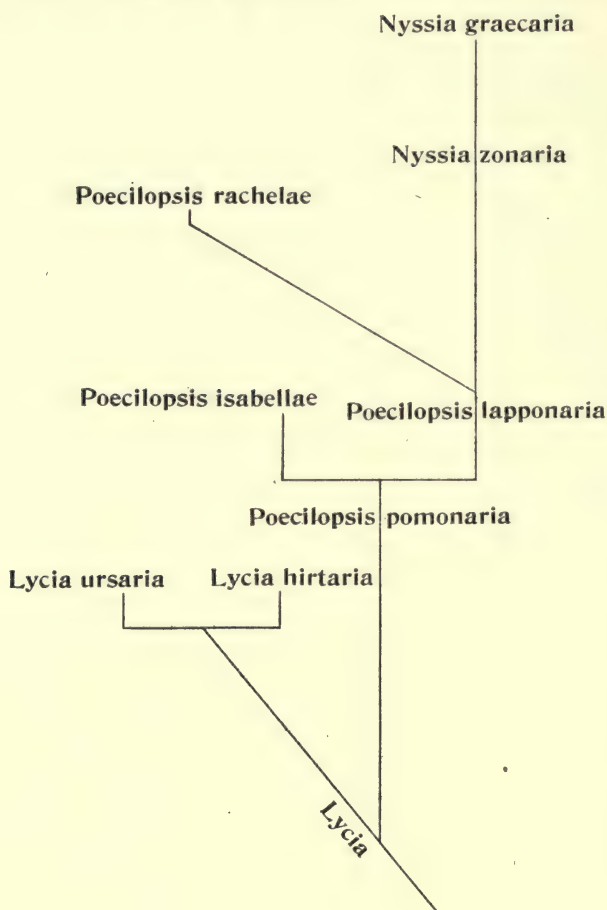
Whether the phrase be restricted to the actual cross fertilisation of plants or extended naturally, as everyone understands, to include the act of avoiding inbreeding in animals, some explanation of the phenomenon must exist, and it is now proposed to consider what light hybridisation experiments in the *Bistoninae* throws on the matter.

Almost immediately the experiments were initiated it was discovered that the hybrid larvae were not only emphatically more robust than those of the weaker parent, but they also surpassed in strength and vitality those of the stronger form. For instance, attempts repeated year by year to rear *Nyssia graecaria* have uniformly failed; yet when this species was mated with *Lycia hirtaria* the resulting larvae were so sturdy and strong that, as larvae, their mortality rate was negligible and, moreover, they fed up in the amazingly short period of six weeks, thereby anticipating the pupation of the more vigorous parent by no less than four weeks. Nor must this be deemed an isolated or exceptional case; to a greater or less degree it illustrates the condition of all the crosses.

Since there were differences in these particulars, as soon as the set of experiments was completed, an endeavour was made to correlate the degree of sturdiness and acceleration of development with other facts, and this had striking success. Taking the ring of hybrids in which *Lycia* figures as the central genus, not only because that circle is practically complete, but also because the crosses concerned in it had

been reared with elaborate care, during the same season, and under conditions likely to eliminate phenotypic variation in all its phases, for another purpose, it was perceived that, as the phylogenetic divergence of the second species in any particular cross from *hirtaria* increased, there was a concomitant and proportional increase in the physiological robustness of the hybrid organism which, in its turn, entailed:

- (1) A size increased beyond the theoretical expectation.
- (2) An acceleration in the speed of feeding up of the larvae.
- (3) Great disease resisting powers.
- (4) An enormous reduction of the time of lying over indulged in by some of the species and which, presumably, should have affected any hybrid in which that species took part.



These correlations can be simplified and rendered more vivid by the appended table and graph, but, before giving these, it will prove very helpful to give a phylogenetical tree of the species involved.

Before proceeding to emphasise the most salient of the relations graphically, it will be well to expand and elucidate the points referred to in paragraph 4 above and placed in the last column of the appended tabular statement.

Female parent	Wing expanse of corresponding male	Wing expanse of <i>hirtaria</i> male	Mean of last two	Wing expanse of the male hybrid between last two	Increase of hybrid wing expanse over mean	Percentage increase over mean	Order of pupation	Effect of hybridity on lying over powers of pupae
<i>Poecilopsis pomonaria</i>	32 mm.	42.5 mm.	37.25 mm.	38 mm.	.75 mm.	2	Last	Nil
<i>Poecilopsis isabellae</i>	31.5 mm.	42.5 mm.	37 mm.	38.4 mm.	1.4 mm.	3.6	Fourth	Slight
<i>Poecilopsis lapponaria</i>	32 mm.	42.5 mm.	37.25 mm.	39.5 mm.	2.25 mm.	6	Third	Great
<i>Nyssia zonaria</i>	29.7 mm.	42.5 mm.	36.1 mm.	39 mm.	2.9 mm.	8	Second	
<i>Nyssia graecaria</i> *	35.25 mm.	42.5 mm.	38.8 mm.	40.1 mm.	1.3 mm.	3.3*	First	Great

* Not reared in the same season and under the same environment conditions as the others.

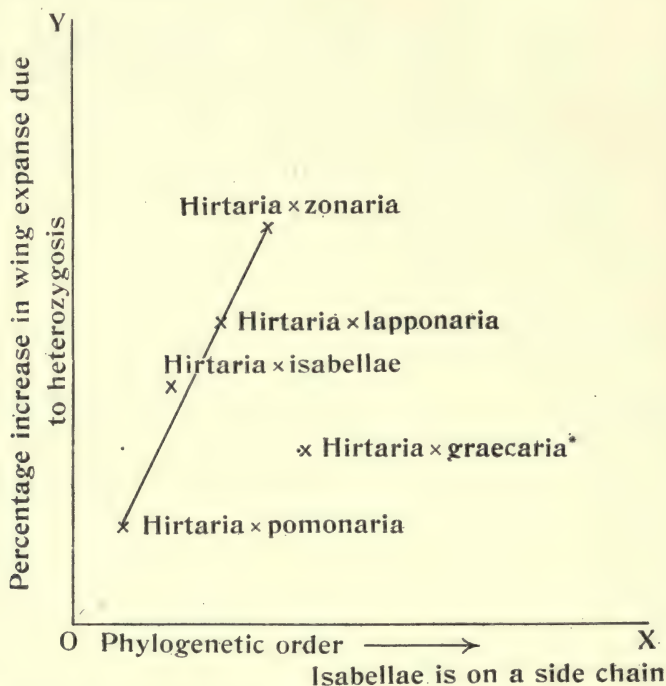
All of the Bistonine species, but more particularly the Non-Boarmioid section, which is of more interest to us at the present juncture, instead of emerging from the pupae together in the spring following pupation, agree, inasmuch as percentages, varying with the species, persist in lying over as pupae from two to eight winters. Of the species here considered, *Nyssia graecaria* is the most notorious offender as it rarely, if ever, emerges in the second year, but commences by providing small detachments in the third year, followed by similarly small instalments each season up to the eighth. *Lapponaria* is the next worst culprit; a limited number appear in the second year, the remainder appearing at intervals in the ensuing three years. *Nyssia zonaria* is extremely erratic in the actual numbers lying over, but all come out before the third spring; *Poecilopsis isabellae* is much the same, whilst in *Lycia hirtaria*¹ and *Poecilopsis pomonaria* nearly all are bred when expected, a very few remaining unchanged until the third year.

And the effect of hybridity is that the further the habit, in this respect, of the second species in the cross from *Lycia*, the greater the acceleration in emergence, and the nearer its habit approaches *L. hirtaria* the less the state of affairs is altered, e.g. cross *Lycia hirtaria* and *Nyssia graecaria* or *L. hirtaria* and *Poecilopsis lapponaria* and all emerge the

¹ Except extreme northern races which were not employed.

first year; however, mate *L. hirtaria* and *P. pomonaria* and the conditions obtaining in the two parent forms are unchanged, the same percentage exactly lying over.

With this explanation, we can now proceed to plot our graph showing the relationship between the phylogenetical divergence of the female parent from *L. hirtaria* and the increased vigour of the hybrid organism as expressed in its size.



* This brood was not reared with the elaborate precautions taken in the other cases to avoid phenotypic variation as, for obvious reasons, it is impossible to get new material from Carniola.

The correlation between the two conditions is so exact as to give as the curve of relationship a straight line.

In other words, the stimulus given to the organism by hybridity is inversely proportional to the physiological affinity of the participating species or, otherwise, it is directly proportional to the physiological divergence, and the same holds true of all the other manifestations of inciting force. Had mere "cross fertilisation" (to adapt the word to include the fusing of gametes the affinities of which are more or less remote) in itself been the sole inducing cause, it is exceedingly difficult

to see how there should have arisen this *progressive* stimulation because, when all is said and done, all the species employed are divided by precisely the same number of generations from the main *Lycaea* stock. It must, therefore, be directly dependent on the cumulative differences between the factors building up the various genotypes (or biotypes, if preferred).

But how have these species arisen and how do they differ? Whether they have been evolved by geographical isolation or by mutation their difference from their immediate phylogenetic ancestor appeared as a change in the value of the genes either by loss, addition, duplication or otherwise; whence it is evident that, as we pass from the *Lycaea stem* to the furthest removed form (physiologically and phylogenetically), we have, *pari passu*, accompanying differences in what were originally homologous genes plus the appearance of genes not represented in the original stock. Therefore, if we pair *L. hirtaria* with any of its derived forms, we are generating zygotes the degree of heterozygosity of which depends entirely upon the extent of the divergence of the second form from *hirtaria*. And this corollary must follow therefrom, that the increased and progressive metabolic action visible in the hybrid series is directly and positively correlated with the advance in the heterozygosity of the individual hybrid members.

Necessarily, of course, a point must be reached when, in the end, total or partial incompatibility¹ of the two sets of chromosomes involved will step in carrying with it, in some, physiological interference with the normal development of the zygote and, in others, the total failure of fertilisation; thus limits are set to the heterozygotic acceleration of the activities of an organism by means of hybridity.

As to how the influence of the heterozygosity works there are several possibilities open. Firstly, one must take cognisance of the fact that any intruding spermatozoon consists of little but the nuclear structures which, coming into intimate contact with the cytoplasm of the ovum, are so placed as to be able to act, react, and interact with that cytoplasm which presents it with entirely novel conditions of environment; thus the stimulus may be brought about. Secondly, the heterogeneous nature of the zygote, the consequent conflicting tendencies of its genes, the extra work induced by the interaction, all combine to secure the laying down of great stocks of cytoplasm and stimulate to an extraordinary degree cell-division, not only in the actual number of cells, but in the speed with which they are elaborated. On this view,

¹ As when *Lycaea hirtaria* ♂ and *Poecilopsis rachelae* ♀ are mated.

the greater the number of diverse determiners (so long as they are able to work without upsetting the stability of the cell thereby causing it to perish) the greater the stimulating effect.

Again, the stimulus may result mainly from the presence in any given cell of a greater number of units than it was designed to receive, causing a development of increased capacity to allow for these extra units, and thus increased and more rapid growth.

In connection with this, it is well to note that, in reality, the size of the cells in the only hybrid I have critically examined was actually greater than that of either *hirtaria* or *zonaria*, the two parents.

Lastly, it must be remarked that the Mendelian factors are not the only reacting features; there are assuredly many nuclear elements not of that type, although such, very likely, predominate, and the activities of this element and the resulting impulses must have some effect.

It will be clear that, up to this stage, only heterozygotic stimulus, as it appears in the F_1 generation of hybrids, has been considered; the reasons for this are plain. Firstly, the F_1 generations in the hybrid *Bistoninae* are, for the most part, completely sterile, and, secondly, each and every zygote generated in any particular cross possesses the same intensity of heterozygosis, not only in the actual number of the opposing factors, but in their quality. Every gamete thus is stimulated to exactly the same extent, and, if we make due allowance for that variation which would be exhibited even by a pure line, will reach approximately the same size in the same time.

But when, as in the *pomonaria-isabellae* hybrids, the F_1 generations are fertile, a new set of circumstances arises on pairing these *inter se*; circumstances, let it be emphasised, not those appearing in F_2 insects of a mono-Mendelian hybrid origin or of hybrids differing by a relatively small number of opposing genes, but those developing in an F_2 generation in which the interacting and conflicting genes reach enormous figures. How soon, under these conditions, the number of heterozygotes reaches appalling dimensions, and how quickly the homozygotes descend to negligible figures may be gathered from the following table:

Number of opposed genes in the parents	Number of individuals	Number of homozygotes	Number of heterozygotes	Percentage of homozygotes
1	4	2	50	50
2	16	4	12	25
3	64	8	56	12.5
4	256	16	240	6.25
5	1024	32	992	3.12
n	4^n	2^n	$2^n (2^n - 1)$	$\frac{100}{2^n}$

Nor is it to be supposed that all of the heterozygotes are heterozygous to the same degree, for that is far from being so. They proceed from monohybrids, through every stage of matroclinous and patroclinous complexes, right up to the full heterozygous complement of the F_1 fraternity.

Hence the stimulating impulse of heterozygosity is felt with unequal force in the different zygotes, and they attain vastly different sizes. Thus, as in characteristics so in size, the F_2 generation shows enormous variation as compared with the comparatively even size and appearance of the F_1 lots. This state of affairs was displayed vividly in the F_1 and F_2 generations of the *pomonaria-isabellae* crosses.

Manifestly therefore, by the mere stimulus of their heterozygosis, independently of any action of multiple genes for size and weight, there will be apparent size segregation in the F_2 insects. Any attempted genetic analyses for size purposes which fail to allow for heterozygotic impulses are vitiated and useless.

To illustrate by examples, size and weight experiments have been undertaken with guinea-pigs, poultry, rabbits, etc. In several of these, dwarf strains of special races have been mated with full-sized examples of other breeds, thereby bringing into play unknown numbers of genes resulting in the production of an equally uncertain number of heterozygotic composites in the F_2 animals carrying with them undetermined and indeterminable degrees of size acceleration. How can it be possible with this unknown factor interfering to conduct any analysis of size and weight determiners?—hence my opinion, expressed above, that many of these analyses are useless. They are not only valueless because heterozygosis will produce size groups of its own, but also because its effects, added to those of the size and weight determiners themselves, serve to throw accelerated specimens into groups to which on the merits of possible weight and size factors they do not belong.

SEX INHERITANCE IN *PEDICULUS HUMANUS* VAR. *CORPORIS*¹.

BY EDWARD HINDLE, M.A., PH.D.,

*Charles Kingsley Lecturer and Bye-Fellow of Magdalene
College, Cambridge.*

(From the Quick Laboratory, Cambridge.)

(With One Chart.)

THE observations on the inheritance of sex in *Pediculus humanus* var. *corporis*, which form the subject of the following pages, were originally commenced in the winter of 1912, when the writer made some experiments on rearing these parasites in connection with the transmission of certain infections. In the course of these experiments it was observed that, under the same conditions, very unequal numbers of males and females were obtained, and, therefore, individual pairs were isolated and their offspring reared separately. It was then discovered that three kinds of families might be obtained, namely, male, female, or male and female.

Many series of experiments were begun, but only two of them were carried through more than one generation, as the conditions of rearing were not understood. Eventually one series was carried through to the second generation, and another one to the fifth, the latter having to be discontinued at the outbreak of war. The results obtained, therefore, are incomplete, and much work remains to be done on the subject, but as the writer is unable to pursue this line of investigation at present, the records of the experiments are being published in the hope that they may be of assistance to students of the inheritance of sex.

In my absence Professor Nuttall kindly published a short account of some of these experiments in *Parasitology* (Hindle, 1917), but unfor-

¹ The expenses of this Investigation were defrayed, in part, by a Grant from the Royal Society.

unately certain errors have occurred in that article, and it is very difficult to trace the histories of the various families. The present account contains a more complete record of the experiments, and also corrections of the former paper.

The cytological examination of fertilization in *Pediculus* has been undertaken by Dr Doncaster, who is continuing the study of the sex problem presented by this parasite.

Material.

In all cases the lice used were collected from verminous clothing sent from London by Dr Hamer. The clothes-lice for any particular series of experiments came off the same piece of cloth, and presumably from the same host. In all cases they belong to the race *Pediculus humanus* var. *corporis*, and there was no evidence of the presence of var. *capitis*, although the possibility of the occurrence of hybrids between these two races should not be ignored, especially in view of the results obtained by Bacot (1917), and Keilin and Nuttall (1919) on hybridisation in lice.

On the arrival of a batch of lice, the larvae were removed from the adults and placed on a piece of black cloth in a glass tube. This was kept in an incubator at a temperature of about 30°C. and the lice were fed twice daily, morning and evening, on the arm¹.

As soon as an adult emerged it was removed to a separate tube, in order to avoid the possibility of any unknown crosses with other emerging adults, and in all cases where a female was found in the same tube as a male, it was not used for any breeding experiments.

A selected male and female were then placed together in the same glass tube and provided with a small piece of black cloth, on which to hold and to lay eggs, and, except in the case of a few experiments, were kept in the same tube and fed together until they died.

When the lice were placed on the arm they usually began to feed at once and gorged themselves in a few minutes. The female commenced to lay eggs about 24 hours after emerging from the larval skin, and generally continued to lay 2 to 4 eggs daily for about four weeks. It should be emphasised, however, that the number of eggs laid by a female fed only twice daily is considerably less than the number

¹ This method has now been improved upon by Nuttall (1917) who obtains much better success with his "pill-box method," whereby the parasites may be kept continually on the body.

occurring when individuals are kept continually on the body, under which conditions as many as 9·7 daily have been recorded.

In my experiments the female rarely laid more than 80 eggs all told, and in many cases considerably fewer, so that the conditions must be regarded as having been somewhat unfavourable.

The larvae usually hatched from the eggs after 10 or 11 days, and moulted three times before becoming adult. The first moult generally occurred after 6 days, and the two following each after 5 days, so that the total development from first stage larva to adult usually took about 16 days. There was a certain amount of mortality amongst the larval stages, as shown in the records on pp. 270—271, where the number of larvae emerging is shown in each case.

When feeding such a large number of lice it was sometimes necessary to place two or three broods on the arm at the same time, but whenever possible they were fed successively, in order to avoid the possibility of their getting mixed. In one or two instances, however, it is probable that individuals have wandered from one brood to another, as shown by the counts of the larvae before and after feeding. In all these cases the experiment is marked with a query.

Immediately an adult appeared it was isolated, and if used for further experiments was crossed with one of the opposite sex as soon as possible. For practical reasons the adults could not be kept alive until all the members of the brood had completed their development, and in consequence some of the crosses had to be made with material that was available rather than that which one would have selected. In view of the onerous task of feeding, all adults not intended for further experiments were killed and preserved, in order to keep down the numbers to a minimum.

The records of these experiments are summarised in the following table, in which is shown (1) the number of the family, *e.g.* B 3 or L 15; (2) the nature of the female parent, *e.g.* ex A 3 signifies that the female came from the brood of family A 3; (3) the nature of the male parent similarly expressed; (4) the total number of larvae that hatched; and (5) the number of each kind of sex among the adults which reached maturity.

In some instances the same male, or the same female, was used for more than one experiment, and these are indicated by footnotes.

RECORDS OF EXPERIMENTS.

First Series.

Number of family	Female parent	Male parent	Number of larvae hatching	Number and sex of adults reared
¹ A 1	Ancestry unknown	Ancestry unknown	18	9 ♂ ♂
¹ A 1a	" "	" "	?	1 ♀ and 2 ♂ ♂
A 2	" "	" "	17	12 ♂ ♂
A 3	" "	" "	20	5 ♀ ♀ and 10 ♂ ♂
A 4	" "	" "	35	26 ♂ ♂
² A 5	" "	" "	5	4 ♀ ♀
A 6	" "	" "	48	32 ♀ ♀
A 7	" "	" "	29	8 ♀ ♀ and 17 ♂ ♂
B 1	ex A 3	ex A 3	36	3 ♀ ♀ and 14 ♂ ♂
B 2	ex A 3	ex A 2	53	34 ♀ ♀
B 3	ex A 3	ex A 2	64	48 ♀ ♀
B 4	ex A 1a	ex A 1a	—	1 ♂

An accident to the incubator prevented any further experiments with this series.

¹ The same individual male was used in both these experiments.

² In this experiment two females and two males were kept together.

Second Series.

Number of family	Female parent	Male parent	Number of larvae hatching	Number and sex of adults reared
L 1	Ancestry unknown	Ancestry unknown	45	27 ♂ ♂
L 2	" "	" "	58	45 ♂ ♂
L 3	" "	" "	38	31 ♀ ♀ and 1 ♂ (?)
L 4	" "	" "	16	9 ♀ ♀
L 8	" "	" "	42	18 ♀ ♀ and 13 ♂ ♂
L 9	" "	" "	9	2 ♀ ♀
L 11	" "	" "	39	28 ♂ ♂ and 2 ♀ ♀
L 12	" "	" "	19	9 ♀ ♀ and 2 ♂ ♂
L 13	" "	" "	46	38 ♂ ♂
¹ L 15	" "	" "	9	6 ♀ ♀
¹ L 15a	" "	" "	50	24 ♀ ♀
L 16	" "	" "	66	64 ♀ ♀
L 17	" "	" "	42	27 ♀ ♀ and 2 ♂ ♂
L 18	" "	" "	25	8 ♂ ♂ and 2 ♀ ♀
L 19	" "	" "	39	26 ♀ ♀
M 1 ex L 19 ex L 18	33	2 ♂ ♂
M 2 ex L 8 ex L 8	12	4 ♀ ♀ and 1 ♂
M 3 ex L 19 ex L 8	Nil	Sterile
M 4 ex L 15a ex L 13	45	36 ♀ ♀
M 5 ex L 8 ex L 13	Nil	Sterile
M 6 ex L 3 ex L 13	20	10 ♂ ♂
¹ M 8 ex L 8 ex L 18	12	3 ♀ ♀ and 4 ♂ ♂
¹ M 8a ex L 8 ex L 18	Nil	—
M 10 ex L 8 ex L 13	16	3 ♀ ♀ and 5 ♂ ♂
M 11 ex L 19 ex L 13	27	5 ♀ ♀ and 4 ♂ ♂
M 12 ex L 19 ex L 13	19	2 ♀ ♀
M 13 ex L 19 ex L 18	19	1 ♀

¹ The same male was crossed with two successive females.

Second Series—(continued).

Number of family	Female parent	Male parent	Number of larvae hatching	Number and sex of adults reared
N 2	ex M 4	ex M 6	78	54 ♂♂
N 3	ex M 4	ex M 6	39	28 ♂♂
N 4	ex M 10	ex M 6	19	6 ♂♂ and 6 ♀♀
N 5	ex M 4	ex M 6	56	42 ♂♂
N 6	ex M 8	ex M 6	31	8 ♂♂ and 12 ♀♀
N 7	ex M 10	ex M 6	38	7 ♂♂ and 15 ♀♀
N 8	ex M 2	ex M 6	30	7 ♂♂ and 10 ♀♀
N 9	ex M 4	ex M 8	43	32 ♂♂
N 10	ex M 4	ex M 8	Nil	Sterile
N 13	ex M 4	ex M 10	47	37 ♂♂
N 14	ex M 4	ex M 10	51	37 ♂♂
N 17	ex M 4	ex M 11	36	12 ♂♂ and 1 ♀ (?)
O 1	ex N 5	ex N 2	Nil	Sterile
¹ O 2	ex N 8	ex N 2	5	1 ♀
O 4	ex N 5	ex N 2	37	13 ♂♂ and 3 ♀♀
¹ O 6	ex N 13	ex N 3	30	14 ♀♀
² O 7	ex N 5	ex N 2	16	3 ♂♂
O 8	ex N 5	ex N 2	36	11 ♀♀
² O 14	ex N 7	ex N 2	29	9 ♀♀ and 1 ♂ ³ (?)
O 16	ex N 7	ex N 2	18	3 ♀♀
O 17	ex N 5	ex N 2	24	2 ♀♀
¹ O 18	ex N 13	ex N 2	10	3 ♀♀ and 1 ♂
	(O 6)	(O 2)		
² O 19	ex N 7	ex N 2	11	1 ♀ and 4 ♀♀
	(O 14)	(O 7)		
P 2	ex O 7	ex O 19	4	1 ♀
P 3	ex O 7	ex O 19	15	3 ♀♀ and 1 ♂
P 13	ex O 18	ex O 6	8	4 ♀♀

¹ The male ex N 2 of O 2 was also crossed with female ex N 13 of O 6 (Family O 18).

² The male ex N 2 of O 7 was also crossed with female ex N 7 of O 14 (Family O 19).

³ This ♀ may have strayed from O 16, as a larva from O 16 was accidentally included with O 14.

A glance at the above records will show that there are at least three distinct types of families, male, female, or mixed. Therefore, four kinds of crosses are possible, namely,

- A. ♀ ex female family × ♂ ex male family.
- B. ♀ " " × ♂ ex mixed family.
- C. ♀ ex mixed family × ♂ ex male family.
- D. ♀ " " × ♂ ex mixed family.

These four types of crosses will be considered separately (see Tables I—IV).

TABLE I.

Female from a female family × male from a male family.

Number of family	Female parent		Male parent	Number of females	Number of males
<i>M</i> 4	ex <i>L</i> 15a	×	ex <i>L</i> 13	36	—
<i>M</i> 6	ex <i>L</i> 3	×	ex <i>L</i> 13	—	10
<i>M</i> 11	ex <i>L</i> 19	×	ex <i>L</i> 13	5	4
<i>M</i> 12	ex <i>L</i> 19	×	ex <i>L</i> 13	2	—
<i>N</i> 2	ex <i>M</i> 4	×	ex <i>M</i> 6	—	54
<i>N</i> 3	ex <i>M</i> 4	×	ex <i>M</i> 6	—	28
<i>N</i> 5	ex <i>M</i> 4	×	ex <i>M</i> 6	42	—
<i>O</i> 4	ex <i>N</i> 5	×	ex <i>N</i> 2	10	3
<i>O</i> 6	ex <i>N</i> 13	×	ex <i>N</i> 3	—	14
<i>O</i> 7	ex <i>N</i> 5	×	ex <i>N</i> 2	3	—
<i>O</i> 8	ex <i>N</i> 5	×	ex <i>N</i> 2	—	11
<i>O</i> 17	ex <i>N</i> 5	×	ex <i>N</i> 2	—	2
<i>O</i> 18	ex <i>N</i> 13	×	ex <i>N</i> 2	3	1
<i>O</i> 1	ex <i>N</i> 5	×	ex <i>N</i> 2	Sterile	

A. All three kinds of families have been obtained. Excluding from consideration those broods in which the number of adults raised is less than four, it will be noticed that there are two female, five male, and three mixed broods, but in the latter there is a decided preponderance of females over males, viz. 18 to 8. Another noteworthy result is that obtained in *N*2, *N*3, and *N*5, where crosses between adults from the same families in two cases produced male broods, and in the other, a female one. It is evident, therefore, that there must be two kinds of males, or two kinds of females, or two kinds each of both sexes.

TABLE II.

Female from a female family × male from a mixed family.

Number of family	Female parent		Male parent	Number of females	Number of males
<i>M</i> 1	ex <i>L</i> 19	×	ex <i>L</i> 18	—	2
<i>M</i> 3	ex <i>L</i> 19	×	ex <i>L</i> 8	Sterile	
<i>M</i> 13	ex <i>L</i> 19	×	ex <i>L</i> 18	1	—
<i>N</i> 9	ex <i>M</i> 4	×	ex <i>M</i> 8	32	—
<i>N</i> 10	ex <i>M</i> 4	×	ex <i>M</i> 8	Sterile	
<i>N</i> 13	ex <i>M</i> 4	×	ex <i>M</i> 10	37	—
<i>N</i> 14	ex <i>M</i> 4	×	ex <i>M</i> 10	37	—
<i>N</i> 17	ex <i>M</i> 4	×	ex <i>M</i> 11	12	1 (?)
<i>P</i> 2	ex <i>O</i> 7	×	ex <i>O</i> 19	1	—
<i>P</i> 3	ex <i>O</i> 7	×	ex <i>O</i> 19	3	1

B. It is unfortunate that the one certainly mixed brood (*P*3) contains but four individuals. The result, such as it is, resembles that obtained in the preceding series, as regards the preponderance of females over males in the mixed broods. With regard to the other

broods, it will be noticed that there is a very decided preponderance of females, the single male family (*M1*) being composed of only two individuals, which are too few to be considered.

TABLE III.

Female from a mixed family × male from a male family.

Number of family	Female parent		Male parent		Number of females	Number of males
<i>B</i> 2	ex <i>A</i>	3	×	ex <i>A</i> 2	34	
<i>B</i> 3	ex <i>A</i>	3	×	ex <i>A</i> 2	48	
<i>M</i> 5	ex <i>L</i>	8	×	ex <i>L</i> 13	Sterile	
<i>M</i> 10	ex <i>L</i>	8	×	ex <i>L</i> 13		
					3	5
<i>N</i> 4	ex <i>M</i>	10	×	ex <i>M</i> 6	6	6
<i>N</i> 6	ex <i>M</i>	8	×	ex <i>M</i> 6	8	12
<i>N</i> 7	ex <i>M</i>	10	×	ex <i>M</i> 6	7	15
<i>N</i> 8	ex <i>M</i>	2	×	ex <i>M</i> 6	7	10
<i>O</i> 2	ex <i>N</i>	8	×	ex <i>N</i> 2	—	1
<i>O</i> 14	ex <i>N</i>	7	×	ex <i>N</i> 2	1	9
<i>O</i> 16	ex <i>N</i>	7	×	ex <i>N</i> 2	3	—
<i>O</i> 19	ex <i>N</i>	7	×	ex <i>N</i> 2	1	4
<i>P</i> 13	ex <i>O</i>	18	×	ex <i>O</i> 6	4	—

C. Excluding from discussion the two families containing less than four individuals, all the families are mixed with the exception of three female broods. There is, however, a distinct preponderance of males over females in the mixed broods, the respective totals of the two sexes being 61 male and 33 female.

TABLE IV.

Female from a mixed family × male from a mixed family.

Number of family	Female parent		Male parent		Number of females	Number of males
<i>B</i> 1	ex <i>A</i>	3	×	ex <i>A</i> 3	3	14
<i>M</i> 2	ex <i>L</i>	8	×	ex <i>L</i> 8	4	1
<i>M</i> 8	ex <i>L</i>	8	×	ex <i>L</i> 18	3	4
<i>B</i> 4	ex <i>A</i> 1a	×	ex <i>A</i> 1a	—	—	1

D. The results of this series are too few for drawing any definite conclusions, but it is suggestive that the three families are all "mixed" broods.

Order in which the adults hatched.

In none of the experiments was there any suggestion that the female first laid eggs of one sex, and towards the end of her life, when exhausted, laid eggs of the other sex. With regard to the mixed broods, the males

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and females alternated fairly regularly in the order of their emergence from the third stage larvae. Records of the order of hatching were kept in each case and the following example is typical of the manner in which the sexes alternated:

Family N 6. ♀ ♂ ♂ ♀ ♂ ♂ ♂ ♀ ♂ ♂ ♂ ♂ ♀ ♀ ♀ ♀ ♂ ♂ ♀.

Sterility.

Although it was not very uncommon to meet with cases in which pairs had no offspring, it is probable that most of them could be explained by the assumption that one or other of the parents was unhealthy and thereby prevented from performing any sexual functions. However, one pair (*N 10*) seems to constitute an example of mutual sterility, for unlike most of these cases, the female laid as many eggs as an ordinary fertile individual, and none of them showed any signs of development. When an unfertilized female is isolated it usually lays a few shrivelled eggs, but the numbers are very much less than in the case of a fertilized individual. It is possible, of course, that in the case mentioned, the mere mechanical effect of the act of copulation may have stimulated the process of egg laying, but there was such a well-marked difference in the numbers and appearance of the eggs laid, that it seems more reasonable to interpret it as an example of sterility.

The same male crossed with two different females.

Three experiments were made in order to determine whether the same male produced the same type of family when crossed with different females. Families *A 1* and *A 1a* constitute the first example, from which it appears that when crossed with one female only male offspring were produced, whilst when crossed with another, a mixed family was obtained. Families *O 2* and *O 18* form another example, but unfortunately only one adult, a male, was reared from the first cross, whilst the second cross resulted in one male and three females. The results, therefore, of this experiment are inconclusive. In a third experiment, families *O 7* and *O 19*, the first cross resulted in three females, whilst the second one produced four males and one female, but in the two latter cases the results are complicated by the fact that the female had also been crossed with another male, as mentioned below. Nevertheless, the result of the first example is supported by the others, and strongly suggests the existence of two types of females in the body louse.

The same female crossed with two males.

In the case of family *O 6* the female was kept with a male for 15 days, during which it laid a certain number of eggs, from which a male brood of 14 males was reared. The female was then isolated for 2 days and afterwards crossed with a different male, the result being shown in family *O 18*. The first adult of this brood to hatch was a male and then followed the three females.

In a second experiment, *O 14* and *O 19*, a female produced the same type of family when crossed with two different males.

These results are complicated by the fact that a female may lay fertile eggs for many days after being isolated from any male, and this should be remembered when considering the results of the first experiment. In this example there is a strong suggestion of the existence of two types of males, for the first individual was only male-producing, whilst the second produced females. With regard to the single male that was the first to hatch in *O 18*, it is possible that it may have grown from an egg fertilized by the first male parent, but in any case, there is a qualitative difference between the two broods.

Hermaphroditism.

In view of the possible existence of hybrids of *P. humanus* var. *corporis* with the variety *capitis*, the male families in the above series of experiments have been carefully examined to see if any individuals showed any signs of hermaphroditism, a feature which is especially characteristic of hybrid races, especially in the males. In family *N 3* one specimen exhibited a slight anal process which Dr Keilin informs me is an indication of this condition, but apart from this doubtful exception, no other individuals have been found which are not normal in this respect. The results of these experiments cannot be explained, therefore, by the assumption that parents from different races were employed, for, as previously mentioned, the original parents were all typical *corporis*, and in Series 1 and 2 came from the same piece of clothing, where they must have lived side by side.

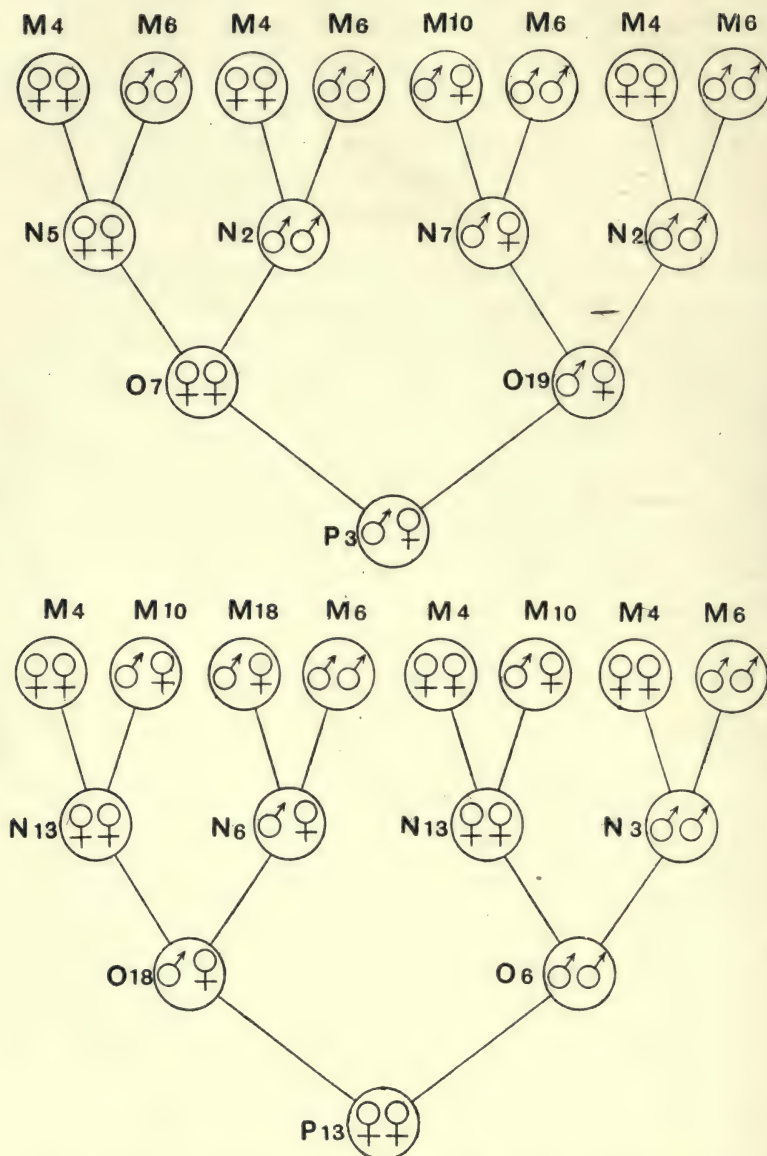


Chart showing the ancestry of families *P3* and *P13*. The nature of the family of the parent is indicated in each case, ♀ ♀ signifying a female, ♂ ♂ a male, and ♂ ♀ a mixed family. The experimental number of the family is also given. Of each pair, the female parent is on the left and the male on the right.

SUMMARY.

1. The results of these experiments show that in *P. humanus* var. *corporis* three types of families may occur:

- (a) Female families,
- (b) Male families,
- (c) Mixed families, in which there is an indication of some ratio between the numbers of the two sexes.

2. There is a strong suggestion of the existence of two kinds of females, as well as two kinds of males.

3. The attached chart gives the pedigree of two families carried through four generations.

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COLOUR INHERITANCE IN CATS, WITH SPECIAL REFERENCE TO THE COLOURS BLACK, YEL- LOW AND TORTOISE-SHELL.

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I. INTRODUCTORY.

This paper has two objects: (1) the critical examination of experimental data on, and of current hypotheses concerning the inheritance of black, yellow, and tortoise-shell coat colours in cats; (2) the suggestion of possible explanations for the occurrence of (a) unexpected colour classes in ordinary crosses between blacks, yellows, and tortoise-shells, and of (b) both sterile and fertile tortoise-shell males which appear extremely rarely.

The fact that the work of all investigators of this subject has left the two points above mentioned not satisfactorily accounted for justifies an attempt to explain the observed experimental results, even though at this time no additional breeding data are offered for consideration.

II. THE FACTS REQUIRING EXPLANATION.

The critical and apparently contradictory facts which have been brought out by breeding experiments with cats, and which must be satisfactorily accounted for and explained, are briefly as follows:

(1) *In crosses between yellow males and black females, where the expectation on the basis of complete sex-linkage is black males and tortoise-shell females, black females are sometimes produced.* (Doncaster, 1913.)

(2) *In crosses between yellow males and tortoise-shell females, where yellow males, black males, yellow females and tortoise-shell females are the only classes expected on the basis of complete sex-linkage, black females are sometimes produced.* (Doncaster, 1913.)

(3) *In crosses between two yellow cats, although only yellow young are expected, two aberrant results have been noted.*

(a) A mating of this type has produced tortoise-shell females besides yellows of both sexes. (Doncaster, 1913.)

(b) A mating of this type has produced tortoise-shell females and black males besides yellows of both sexes. (Whiting, 1918.)

(4) There is no record of two black cats crossed together having given yellow or tortoise-shell young.

(5) *Tortoise-shell males are produced much more rarely than any of the aberrant classes recorded under headings 1, 2, and 3 above.* (Doncaster, 1913; Wright, 1918.)

(6) *Such tortoise-shell males are usually sterile.* (Cutler and Doncaster, 1915.)

(7) *If they are not sterile they apparently do not give tortoise-shell sons, but breed as yellows.* (Doncaster, 1913.)

In considering these facts, investigators have usually tried to explain all of them by a single hypothesis. (Doncaster, 1913; Whiting, 1918.) This has proved to be difficult and unsatisfactory. (Ibsen, 1916; Wright 1918.)

It is believed that the experimental evidence favours the existence of two genetically independent agents at work in the production of these aberrances, for

(a) The appearance of the unexpected individuals noted under headings 1, 2, and 3 above, is relatively frequent, and produces regular results involving neither sterility nor the formation of new colour types.

(b) On the other hand, the occurrence of tortoise-shell males is *very infrequent*, not regular, and is in a majority of cases intimately connected with sterility.

Such being the case, an effort will be made to explain the appearance of the unexpected individuals noted under headings 1, 2, and 3 by one hypothesis and the occurrence of tortoise-shell males by a different one.

III. THE RELATION BETWEEN YELLOW AND BLACK.

One of the first points to be established is the nature of the genetic relation between yellow coat colour and black coat colour.

In this connection Ibsen, 1916, and Wright, 1918, believe black or extension of black pigment to the coat, to be epistatic to yellow or the restriction of black pigment from the coat. Doncaster, 1913, and

Whiting, 1918, consider the two coat colours allelomorphic, the heterozygote being commonly tortoise-shell.

The terminology used by them is as follows:

Ibsen, 1916: Black B is dominant to orange b which is borne in the X chromosome. Under ordinary conditions the factor for orange b is closely linked to T , a factor for tortoise-shell which acts only in the presence of B -black. The female is XX , the male $X\theta$ in formula.

Wright, 1918: Black is due to the action of a factor A , while tortoise-shell is produced by heterozygosis of an "extension" factor E . Tortoise-shell females are thus Ee , yellow males $e-$, and black males $E-$, in formula. The factor E is borne in the X chromosome. The female is XX , the male $X\theta$ in formula.

Doncaster, 1913 considers that yellow and black are allelomorphic, and expresses yellow by Y , and black by B . Where both are present, a YB or tortoise-shell animal is produced. The female is XX , and the male $X\theta$ in formula.

Whiting, 1918 also considers yellow Y to be allelomorphic with black y , and supposes Y to be borne in the X chromosome. The female is homozygous, the male heterozygous for X .

In 1912 I employed much the same terminology as that of Doncaster, but in view of the production of blacks and tortoise-shells by two yellows and the failure of blacks when crossed *inter se* to produce anything except blacks, it is probable that the relationship between these two colours may be more accurately expressed in somewhat the following manner: B a factor for the production of black pigment which is found in all X gametes. Y a factor for the restriction of black pigment from the coat allelomorphic to y , a factor for the extension of black pigment to the coat. One "dose" of Y is normally completely epistatic to one "dose" of B , thus producing yellow individuals; but two "doses" of B to one of Y produces a tortoise-shell. The factor Y and its allelomorph y are also borne in the X chromosome. Thus:

YBX	YBX	Yellow female
YBX	θ	Yellow male
yBX	yBX	Black female
yBX	θ	Black male
YBX	yBX	Tortoise-shell female

This type of relationship will become clear as the crosses are taken up in detail, and is further made use of in explaining the occurrence of tortoise-shell males.

IV. AN ATTEMPT TO EXPLAIN THE APPEARANCE OF UNEXPECTED INDIVIDUALS OF NORMAL COLOUR TYPES. (Headings 1, 2, and 3, Section II, above.)

It is tacitly assumed by all investigators that at some time or times in the past, there must have been a genetic change, ridding certain gametes of the epistatic colour factor, whether it be the Y of Whiting, the E of Wright, or the T of Ibsen. Had this not been the case neither the hypostatic form nor the tortoise-shell heterozygote could have appeared.

We may, then, for the sake of argument accept the set of symbols given above, and assume that the change from Y to y must have occurred. There is no experimental evidence to show how recently or how frequently this change may have taken place, but if we assume that it is still taking place in a portion of the gametes of certain individuals—which seems entirely probable—all the results obtained under headings 1, 2, and 3, may be accounted for. Such a change from an epistatic to a hypostatic condition would be directly comparable to the appearance of the recessive pink-eyed mutation in a stock of dilute brown mice recorded by the writer in 1916.

Animals in whose gametes this mutative process was occurring *de novo* would show no trace of it in their own somatic characteristics, but would, upon breeding, give results in agreement with the actual aberrant classes obtained.

We should thus expect that an occasional yellow female would form gametes yBX in addition to those containing YBX which she normally produces. Similarly, certain yellow males would be found which showed by their progeny that they were forming among their X gametes some which were of the constitution yBX instead of the normal YBX type.

Yellow males of this unusual kind would, when crossed with black females, give among their progeny a certain number of black females, in number depending upon the frequency with which the unusual yBX sperm was formed. This fact would explain the aberrances listed above under Section II, Heading 1.

Similarly, such unusual yellow males would, when mated to normal tortoise-shell females, give rise to a certain number of black females in addition to the other classes normally expected. This would cover category two of exceptions mentioned above (Section II).

Finally, a yellow forming yBX gametes, when crossed with a normal yellow or with one of its own type, would give rise to unexpected black

or tortoise-shell young, the proportion depending upon whether the yellow male or the female or both were concerned in the formation of the yBX gametes.

Thus if the male was alone concerned, tortoise-shell females, but *no black males* would be likely to appear among the progeny. This appears to be the case in the mating recorded by Doncaster (1913) in which two yellows gave among their progeny three blue females with a cream coloured patch (tortoise-shells). If, on the other hand, the female parent was the unusual mutative individual, *black males* would occur in addition to tortoise-shell females and yellows of both sexes. This condition was realised in the case of female dilute yellow #23 (formerly owned by me) whose breeding record is reported by Whiting, 1918. An explanation of this sort would account for the aberrances noted under Section II, Heading 3, above.

From the number of tortoise-shell and black young obtained in the two cases referred to, and from the numerical relation of the black females under headings 1 and 2 (Section II) to the expected colour classes (Doncaster, 1913), it seems probable that yellow animals forming yBX gametes do so in approximately 50% of the gametes they form, as would a normal heterozygote.

In addition to yellow animals, certain tortoise-shell females might theoretically be expected to show the same phenomenon. Such animals would form an excess of, or possibly exclusively, yBX gametes, and, in so far as they did so, would breed as blacks. Such an occurrence would, however, give rise to no unexpected classes of young in crosses, but might result in the *absence* of some of those normally expected from certain matings. Quite naturally this fact might, in a small number of progeny, escape notice.

There is no evidence to show that the appearance of any of the classes above referred to is in any way connected with a break in sex-linkage or with the occurrence of tortoise-shell males, and we may therefore, until such evidence is presented, fairly consider them as independently produced.

V. CRITICISM OF EXISTING HYPOTHESES TO EXPLAIN THE APPEARANCE OF UNEXPECTED INDIVIDUALS OF NORMAL COLOUR TYPES. (Headings 1, 2, and 3, Section II, above.)

Attempts to explain the appearance of the aberrant colour classes referred to, have involved either (a) the breaking of sex-linkage with "crossing over" in the male, or (b) the occurrence of a series of modifying

factors determining the relative degree of black and yellow pigmentation. They may be separately considered as follows:

(a) Doncaster's hypothesis of a break in sex-linkage: this hypothesis, which in a modified form is a basis for Ibsen's later explanation of the appearance of unusual colour types, involves, if it is to explain the exceptional black females, the existence of "crossing over" in the male between the X and the θ chromosomes. Such crossing over has not, in so far as I am aware, been demonstrated in any forms in which the male is $X\theta$ in formula as in cats. It further would suppose that, as tortoise-shell males were formed by the same process, they would be expected to occur with as great frequency as the exceptional black females. It further leaves entirely unexplained the appearance of blacks or tortoise-shells from a cross between yellow animals. These objections seem to be of sufficient weight to throw the chances against Doncaster's or Ibsen's hypotheses.

(b) Whiting's hypothesis of modifying factors which at one end of the series would serve to make tortoise-shell animals yellow, and at the other end of the series make them black, remains as a possibility though seriously invalidated by certain points as follows:

(1) There should be records of black females (genotypically tortoise-shell) which if crossed with other blacks should give yellow males and tortoise-shell females, or if crossed with yellow males should give unexpected yellow females. Neither of these results has been recorded.

(2) Doncaster, 1913, reports that the three tortoise-shell females produced from a single cross between two yellows were "blue with a cream patch" thus showing that they were near the *black* end of Whiting's modifier series. Inasmuch as under his hypothesis one of their parents must have been at the opposite or yellow end of the series, it is difficult to explain how and why many of its progeny should show the condition characterising nearly the other end of a graded series.

(3) The occurrence of these young in a single mating makes it seem likely that the particular animal was forming ordinary yBX gametes in a considerable number.

(4) The tortoise-shell young produced by dilute yellow female #23 already referred to, before she was sent to Dr Whiting, were normal tortoise-shell in colour; if anything, more nearly on the *black* end of the graded series, than on the yellow. This case serves to support that

reported by Doncaster, and tends to show that the yellow animal *transmitted to its progeny no peculiar set of modifiers*.

VI. AN ATTEMPT TO EXPLAIN THE OCCURRENCE OF (a) STERILE, AND (b) FERTILE TORTOISE-SHELL MALES.

(a) *The production of sterile tortoise-shell males.*

It is agreed by all those who have reported on breeding experiments with cats that the female appears to be homozygous, the male heterozygous, for sex. The former is therefore XX , the latter $X\theta$ in formula. This places cats in the same category with *Drosophila*, and this in turn means that one may rightfully turn, and in fact *should* turn, to the magnificent work of Morgan and his associates in any attempt at explaining a peculiar result which shows exceptional conditions of sex-linkage.

If one considers the phenomena of non-disjunction of the X chromosome in *Drosophila*, reported by Bridges in 1913, and later (1916 *a* and *b*) further established by him after an extensive series of breeding experiments, one cannot fail to be impressed by the similarity between the results of that process in *Drosophila*, and the observed experimental facts in cats.

For example, non-disjunction is neither frequent in its occurrence nor is it clear enough in its hereditary behaviour to give striking numerical results in as slow breeding an animal as a cat, unless it were watched for deliberately. In *Drosophila* it gives rise to two very significant exceptions to the normal sex-linked inheritance. First, it produces animals *apparently males, which are sterile*, and second, *mosaic forms* are apt to arise in non-disjunctive stocks. If one considers that the majority of tortoise-shell cats *which are apparently males are sterile*, and second that they are also a *mosaic* form in a sex where commonly none is found, the comparison becomes interesting and extremely suggestive.

We may now consider what the probable results of non-disjunction would be, did this phenomenon exist in cats.

The characteristic of primary non-disjunction is that in oogenesis the two X chromosomes go together into a single egg, leaving another egg without even the normal single X . This may be shown as follows:

Non-disjunctive female XX
forms gametes XX and

If now the eggs of such a female are fertilized by sperm of a normal male we have four possible types of zygotes.

Eggs	Sperm	Zygote	
XX	X	XXX	Dies
-	X	X-	"Near male" always sterile
XX	θ	XX θ	Female with peculiar gametic condition
-	θ	θ -	Dies

Bridges has demonstrated that the XXX and θ - forms die, and that the X- form although appearing like a male is always sterile. If now we imagine a cross to be made between a tortoise-shell female cat showing non-disjunction and a normal yellow male, we should have the following condition:

Non-disjunctional Tortoise-shell female		YBXyBX	Normal Yellow male YBX θ
Forming gametes		YBXyBX and -	Forming gametes YBX and θ
Zygotes	(a)	YBXyBXYBX	Dies
	(b)	YBXyBX θ	Tortoise-shell with peculiar gametic conditions
	(c)	YBX-	"Near male" always sterile
	(d)	- θ	Dies

If now one assumes that absence of the θ chromosome allows the "near male" class (c) to develop into a tortoise-shell, disturbing the normal relation of yellow to black to produce a somatic mosaic, we could account for the appearance at rare intervals of tortoise-shell "near males" which were not fertile. It seems not unlikely that the absence of the θ chromosome might well upset the somatic relationships of certain of the characters whose factors are carried by the X chromosome. This would account for the appearance of a tortoise-shell "male" from a mating of yellow male \times tortoise-shell female. (Doncaster, 1913.)

Another mating which, according to Doncaster, has produced a tortoise-shell male is that of yellow male by black female. Here, if the black female showed non-disjunction, the following condition would be found:

Black non-disjunctional female yBXyBX		Normal yellow male YBX θ
Forming gametes		yBXyBX and -
Zygotes	(a)	yBX yBX YBX Dies
	(b)	yBX yBX θ Black female with peculiar gametic conditions
	(c)	- YBX Tortoise-shell? "near male" always sterile (as in previous mating)
	(d)	- θ Dies

The third type of mating reported by Doncaster as having produced a tortoise-shell male is that of black male with tortoise-shell female. Here everyone is in difficulty. If, as Doncaster suggests, the occasional crossing over of Y, the factor for yellow, to a θ gamete is responsible for

the production of a tortoise-shell male, nothing that could happen in either the gametes of the black male or of the tortoise-shell female would produce a tortoise-shell male. On Whiting's hypothesis we should have to suppose that the tortoise-shell female, although she herself showed no marked modifiers (or she would have been black), transmits unusually heavy modifiers to her sons. These gametes would in turn have to be met by an equally heavy set of modifiers from the black male, or a yellow would result.

Further than this, by Whiting's hypothesis the yellow male is $YX\theta$ in constitution, and this makes the source of the black that he must produce somatically under the influence of modifiers in order to become a tortoise-shell, uncertain. This condition is, of course, not impossible but is highly improbable. Finally, the phenomenon of non-disjunction meets with distinct difficulties. Unless the black male forms gametes with neither the X nor θ chromosomes present it would be hard to see how the tortoise-shell male could be produced by this mating. Formation of sperm without X or θ would not be likely. Yet the possibility exists and may therefore be considered. What seems to me altogether more likely is that the breeder's records on which Doncaster based his observation were in this case uncertain or incorrect, a circumstance quite possible in cats even with the best possible intentions.

(b) *The production of fertile tortoise-shell males.*

We have seen that peculiar tortoise-shell females of formula $YBXyBX\theta$ may possibly be produced by primary non-disjunction. If now one of these females is crossed with a black or a yellow male peculiar yellow males of the constitution $YBX\theta\theta$ would be formed as follows:

Non-disjunctional Tortoise-shell female $YBXyBX\theta$		crossed with	Yellow male $YBX\theta$
Forming gametes	$YBXyBX$, $YBX\theta$, YBX , θ , $yBX\theta$ and yBX	Forming gametes	YBX and θ
Zygotes	(a) $YBXyBXYBX$	Dies	
	(b) $YBX\theta YBX$	Peculiar yellow female	
	(c) $YBXYBX$	Yellow female	
	(d) θYBX	Yellow male	
	(e) $YBXyBX\theta$	Peculiar tortoise-shell female	
	(f) $YBX \theta\theta$	Peculiar yellow male	
	(g) $YBX \theta$	Yellow male	
	(h) $\theta\theta$	Dies	
	(i) $yBX\theta YBX$	Peculiar tortoise-shell female	
	(j) $yBX\theta\theta$	Peculiar black male	
	(k) $yBXYBX$	Tortoise-shell female	
	(l) $yBX \theta$	Black male	

If now such a peculiar yellow, $YBX\theta\theta$, is mated with any female showing primary non-disjunction—an animal which might well prove to be a fertile tortoise-shell male would be produced. Thus:

Non-disjunctional Black female $yBXyBX$		crossed with Non-disjunctional Yellow male $YBX\theta\theta$	
Forming gametes	$yBXyBX$ and —	Forming gametes	$YBX\theta$, YBX , θ and $\theta\theta$
(a)	$yBXyBX$ $YBX\theta$	Dies	
(b)	$yBXyBX$ YBX	Dies	
(c)	$yBXyBX$ θ	Peculiar black female	
(d)	$yBXyBX$ $\theta\theta$	Peculiar black female?	
(e)	— $YBX\theta$	"Tortoise-shell male" fertile?	
(f)	— YBX	"Tortoise-shell 'near male' sterile"?	
(g)	— θ	Dies	
(h)	— $\theta\theta$	Dies	

Here the assumption is made that an animal formed from the combination of gametes, $YBX\theta$ and —, may be somatically a tortoise-shell, and that the θ chromosome which is brought into the zygote by an X -bearing gamete does not in all cases exert its full influence until gametogenesis. The $YBX\theta$ -male would then be supposed to develop somatically just as does the YBX -animal, but upon gametogenesis the θ chromosome of the $YBX\theta$ -male is able to prevent the sterility which exists in its absence. This seems quite possible, for it appears that in *Drosophila* the θ chromosome is not needed for the development of the normal male somatic characters, but that it is necessary, however, for successful gametogenesis in the male.

A fertile tortoise-shell male would, when he formed gametes, behave exactly like a normal yellow male. That is to say, although he was himself the product of a combination of $X\theta$ and — gametes, he would in gametogenesis form only X and θ gametes, just as would a normal male. This has been the breeding behaviour of the one recorded certainly fertile tortoise-shell male (see Doncaster, 1913) which acted in crosses with tortoise-shell females apparently exactly as a yellow male would have done.

It will be seen that the above hypothesis, although somewhat complicated, is nevertheless in accordance with experimental facts and accounts for sterile and fertile types of tortoise-shell males; it explains their infrequency of appearance, and possibly their failure to transmit their own colour pattern to their descendants; it is supported by the work of Bridges with *Drosophila*—the most completely investigated form showing a similar type of sex-linkage; it is further capable of experimental tests.

VII. CRITICISMS OF EXISTING HYPOTHESES TO EXPLAIN THE OCCURRENCE OF TORTOISE-SHELL MALES.

(1) Doncaster's hypothesis, as already pointed out, requires "crossing over" in the male between X and θ chromosomes—a condition not shown to exist in gametogenesis of any $X\theta$ male form. It further fails to account for (a) the comparative infrequency of tortoise-shell males as compared with aberrant black females, (b) the sterility of the majority of tortoise-shell males, and (c) their peculiar behaviour in breeding.

(2) Ibsen's hypothesis does away with the need of crossing over in the male, but fails, as does Doncaster's hypothesis, to meet or explain points (a), (b), or (c) stated above.

(3) Whiting's hypothesis of modifiers would not be able to give a tortoise-shell male which according to his formula would be $XY\theta$ without adding a factor for black to the formula given by him to be carried in the X gamete. It further would suppose that by selection (which undoubtedly has occurred) it would be possible to transmit the necessary modifiers to a considerable number of his male progeny, thus forming tortoise-shell males—and this, though great efforts have been made, has proved impossible. Whiting's hypothesis, like those of Doncaster and of Ibsen, takes no account of the sterility of the majority of tortoise-shell males.

(4) Wright's hypothesis is that tortoise-shell males are really XX individuals in which the abnormality lies not in the colour but in the sex. He likens them to certain sex intergrades already described in some forms by other investigators. This hypothesis meets trouble when a fertile tortoise-shell male is encountered. It also is contrary to the evidence obtained by Bridges who shows that in *Drosophila* XX forms are females, even though they contain other abnormalities of chromosome distribution.

VIII. SUMMARY AND CONCLUSIONS.

(1) The genetic constitution of the normal colour varieties of cats as regards yellow and black pigmentation appears to be as follows: B = a factor producing black pigmentation, Y = a factor which restricts black from the coat, y = a factor allelomorphic to Y and hypostatic to it, allowing black pigment to extend to the coat.

YBX	YBX	Yellow female
YBX	θ	Yellow male
yBX	yBX	Black female
yBX	θ	Black male
YBX	yBX	Tortoise-shell female

(2) The unexpected but normally pigmented individuals appearing in certain matings (Headings 1, 2, and 3, Section II) can be accounted for by supposing that Y becomes y in a certain proportion of the gametes of exceptional individuals.

(3) Sterile tortoise-shell males may possibly be "near males" formed as a result of non-disjunction of the X chromosome and therefore $YBX-$ in constitution.

(4) Fertile tortoise-shell males may also be the product of non-disjunction (secondary) and would be zygotes formed from the fusion of gametes $YBX\theta$ and $-$. These males in gametogenesis would behave as ordinary yellows.

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THE PROBABLE ERRORS OF CALCULATED LINKAGE VALUES, AND THE MOST ACCURATE METHOD OF DETERMINING GAMETIC FROM CERTAIN ZYGOTIC SERIES.

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IN view of the controversy as to the cause of coupling and repulsion it is desirable to know the probable error of any given determination of a gametic ratio, and also to obtain from an observed zygotic series the most accurate possible estimate of the corresponding gametic series. By the probable error of an observation is meant of course a number such that the difference between the true and observed values is equally likely to exceed it, or to fall short of it.

We shall consider the case of a heterozygote $AaBb$ which produces gametes in the proportions $\frac{p}{2} AB : \frac{1-p}{2} Ab : \frac{1-p}{2} aB : \frac{p}{2} ab$. In the case of repulsion p is the cross-over value, in that of coupling $1-p$. If we write the gametic series in the case of coupling as

$$xAB : 1Ab : 1aB : xab,$$

in the case of repulsion as

$$1AB : yAb : yaB : 1ab,$$

then

$$x = \frac{p}{1-p}, \quad y = \frac{1-p}{p}.$$

The values of p , x , and y may be obtained directly from the cross $AaBb \times aabb$. Here if n is the total number of zygotes obtained, P the observed value of p , and (AB) , (ab) the observed numbers of zygotes of compositions $ABab$ and $abab$ respectively, $P = \frac{(AB) + (ab)}{n}$. If n were infinite P and p would of course be equal, actually the probable error of P is

$$.6745 \sqrt{\frac{P(1-P)}{n}} \dots\dots\dots (1).$$

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A direct proof of this result from Bayes' theorem is given by Todhunter(1); the proof generally given is for the probable error when p , the true value, is known beforehand.

More accurately the probable error in excess is

$$\cdot6745 \sqrt{\frac{P(1-P)}{n}} + \frac{\cdot3033(1-2P)}{n} \dots\dots\dots(2),$$

that in defect

$$\cdot6745 \sqrt{\frac{P(1-P)}{n}} - \frac{\cdot3033(1-2P)}{n} \dots\dots\dots(3).$$

This correction however is never important, and may be neglected for all ordinary purposes if both nP and $n(1-P)$ are sufficiently large (say over 100).

Let

$$x = X + \xi, \quad p = P + \pi.$$

$$\begin{aligned} \therefore X + \xi &= \frac{P + \pi}{1 - P - \pi} \\ &= \frac{x + \pi}{(1 - P)^2}, \text{ provided } \pi \text{ is small compared with } P, \\ &= x + (X + 1)^2 \pi. \end{aligned}$$

Hence the probable error of X is

$$\cdot6745 (X + 1)^2 \sqrt{\frac{P(1-P)}{n}},$$

or

$$\cdot6745 (X + 1) \sqrt{\frac{X}{n}} \dots\dots\dots(4).$$

Similarly that of Y is

$$\cdot6745 (Y + 1) \sqrt{\frac{Y}{n}} \dots\dots\dots(5).$$

It may be remarked that when p , x , or y are determined by this method we automatically eliminate the effects of differential mortality due to one factor only, or to both if they affect the mortality independently. If however both factors affect the viability it will generally be safer to employ Morgan and Bridges'(2) "balanced inviability" method.

To take a concrete example of the above calculation, Altenburg(3), working with the factors M and S (Magenta and Green stigma) in *Primula sinensis*, obtained from the cross $MS.ms \times mmss$, from a count of 3684 plants, a value of $\cdot884$ for P . The probable error of this value

is therefore $\cdot6745 \sqrt{\frac{\cdot884 \times \cdot116}{3684}}$, or $\cdot00358$.

Hence the cross-over value is $11.6 \pm .36\%$, $X = 7.59$ and its probable error is $.6745 \times 8.59 \sqrt{\frac{7.59}{3684}}$, or .28. Thus neither 7 nor 8 are improbable values for x , the probability of an error as great as or greater than .59 being only about .27%, as may be seen from a table of values of $\frac{2}{\sqrt{\pi}} \int_0^x e^{-t^2} dt$.

We shall now consider the zygotic series obtained in F_2 by mating *inter se* the F_1 zygotes considered above. The expected proportions are

$$\frac{2+p^2}{4} AB : \frac{1-p^2}{4} Ab : \frac{1-p^2}{4} aB : \frac{p^2}{4} ab,$$

where AB , etc. represent the visible characters of the zygotes. Consider a group of n such zygotes. Put $t = p^2$, and let t_a be an approximate value of t , while $T = t_a + \delta$ is the value which is most probable from the given observation. Also let t_1, t_2, t_3, t_4 be the four values of t calculated from the four classes observed.

$$\text{Then} \quad t_1 = \frac{4(AB)}{n} - 2,$$

$$t_2 = 1 - \frac{4(Ab)}{n},$$

$$t_3 = 1 - \frac{4(aB)}{n},$$

$$t_4 = \frac{4(ab)}{n}.$$

$$\therefore t_1 + t_4 = t_2 + t_3.$$

Hence for any value of δ the probability of the observed proportions

$$\frac{2+t_1}{4} AB : \frac{1-t_2}{4} Ab : \frac{1-t_3}{4} aB : \frac{t_4}{4} ab$$

being obtained in a group of n zygotes is proportional to

$$\text{Exp.} = 2^n \left[\frac{\left(\frac{t_a + \delta - t_1}{4}\right)^2}{\frac{2+t_a+\delta}{4}} + \frac{\left(\frac{t_a + \delta - t_2}{4}\right)^2 + \left(\frac{t_a + \delta - t_3}{4}\right)^2}{1-t_a-\delta} + \frac{\left(\frac{t_a + \delta - t_4}{4}\right)^2}{\frac{t_a + \delta}{4}} \right],$$

or

$$\text{Exp.} = 8^n \left[\frac{(t_a - t_1 + \delta)^2}{2+t_a} + \frac{(t_a - t_2 + \delta)^2 + (t_a - t_3 + \delta)^2}{1-t_a} + \frac{(t_a - t_4 + \delta)^2}{t_a} \right],$$

since δ may be neglected in comparison with t_a or $1-t_a$, though not with $t_a - t_1$, etc.

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When T is the most probable value this probability is a maximum, and hence the expression in brackets is a minimum. The condition for this is

$$\delta = \frac{(t_1 - t_a)(1 - t_a)t_a + (t_2 + t_3 - 2t_a)(2 + t_a)t_a + (t_4 - t_a)(2 + t_a)(1 - t_a)}{(1 - t_a)t_a + 2(2 + t_a)t_a + (2 + t_a)(1 - t_a)}.$$

Hence, putting

$$t_2 + t_3 = t_1 + t_4,$$

$$T = t_a + \delta = \frac{3t_at_1 + (2 + t_a)t_4}{2 + 4t_a} \dots\dots\dots(6),$$

while

$$P = \sqrt{T}.$$

As our value of t_a we may take t_4 in the case of repulsion, $\frac{1}{2}(t_1 + t_4)$ in the case of coupling. If greater accuracy is desired the value of T thus obtained should be substituted for t_a in equation (6), and a more accurate value thus obtained. This proceeding is however rarely worth while.

The same value for T is reached more directly by Bridges' method(5), where T is calculated from the coefficient of association

$$\frac{(AB) \times (ab) - (Ab) \times (aB)}{(AB) \times (ab) + (Ab) \times (aB)} = \frac{4T - 1}{2T^2 + 1},$$

as may readily be seen on substituting the value $\frac{n}{4}(2 + t_1)$ for (AB) , and so on. It is doubtful however if this method is any shorter than that given above, unless a four figure table of values of P in terms of the coefficient of association has been calculated in advance.

To take a concrete example, Punnett(4) working with the coupled factors B and L in sweet peas obtained the F_2 zygotic series

$$4831BL, \quad 390Bl, \quad 393bL, \quad 1338bl.$$

Here $n = 6952,$

$$t_1 = \frac{4 \times 4831}{6952} - 2 = \cdot 7796,$$

$$t_4 = \frac{4 \times 1338}{6952} = \cdot 7699,$$

$$t_a = \frac{1}{2}(t_1 + t_4) = \cdot 77475.$$

$$\therefore T = \frac{3 \times \cdot 77475 \times \cdot 7796 + 2 \cdot 77475 \times \cdot 7699}{2 + 4 \times \cdot 77475} = \cdot 7743.$$

If we substitute $\cdot 7743$ for t_a in equation (6) we obtain no change in the first four decimal places.

Hence $P = \sqrt{T} = \cdot 8800$, and the cross-over value is $12\cdot00$ *bl*,

$$X = \frac{P}{1-P} = 7\cdot333,$$

and the calculated expectation is

$$4821\cdot7BL, \quad 392\cdot3BL, \quad 392\cdot3bL, \quad 1345\cdot7bl,$$

against observed numbers

$$4831 \quad BL, \quad 390 \quad BL, \quad 393 \quad bL, \quad 1338 \quad bl.$$

We have now to calculate the probable errors of the values of T , P , X , and Y obtained above.

The probability of any value $T + \alpha$ of t varies as

$$\text{Exp.} - \frac{n}{8} \left[\frac{(T - t_1 + \alpha)^2}{2 + T + \alpha} + \frac{(T - t_2 + \alpha)^2}{1 - T - \alpha} + \frac{(T - t_3 + \alpha)^2}{T + \alpha} \right].$$

But the coefficient of α in the exponent vanishes, and α may be neglected in comparison with T or $1 - T$ unless n is small, hence the probability varies as

$$\text{Exp.} - \frac{n(1 + 2T)\alpha^2}{4T(2 + T)(1 - T)}.$$

Hence the probable error of T is

$$\cdot 477 \sqrt{\frac{4T(2 + T)(1 - T)}{n(1 + 2T)}}.$$

That of P is $\frac{1}{2P}$ of this, or

$$\cdot 477 \sqrt{\frac{(2 + P^2)(1 - P^2)}{(1 + 2P^2)n}} \dots\dots\dots(7).$$

The probable error of X is

$$\cdot 477(X + 1) \sqrt{\frac{(3X^2 + 4X + 2)(2X + 1)}{(3X^2 + 2X + 1)n}} \dots\dots\dots(8).$$

or, when X is large, approximately

$$\cdot 6745(X + 1) \sqrt{\frac{X + \frac{1}{2}}{n}} \dots\dots\dots(9).$$

That of Y is

$$\cdot 477(Y + 1) \sqrt{\frac{(2Y^2 + 4Y + 3)Y(Y + 2)}{(Y^2 + 2Y + 3)n}} \dots\dots\dots(10).$$

or, when Y is large, approximately

$$\cdot 6745 \frac{(Y + 1)^2}{\sqrt{n}} \dots\dots\dots(11).$$

Comparing these values with the probable errors, given by formulae (1), (2) and (3), of the values obtained by crossing F_1 with the double recessive we see that the latter are always smaller. When P is nearly 1 the accuracies are nearly equal, but when P is small the direct method is $\frac{1}{\sqrt{P}}$ times as accurate as the indirect, i.e. $\frac{1}{P}$ times as many zygotes obtained by the indirect method must be counted, in order that it should give a result as accurate as the direct method. When $P = \frac{1}{2}$, the ratio of the probable errors is only 3:2.

Hence the ratios calculated from F_2 are nearly as reliable as those obtained from $F_1 \times$ the double recessive in the case of coupling or weak repulsion, but with strong repulsion they are somewhat unreliable. Hence Bridges' stricture (5) on the unreliability of values derived from F_2 results is only justified in the case of strong repulsion.

Moreover, since its numbers vary as the square of the cross-over value, the double recessive class in F_2 is a more sensitive indicator of repulsion than any class derived from the cross $F_1 \times$ double recessive. Thus the enumeration of F_2 is somewhat more sensitive as a test for linkage, and about equally accurate as a measure of its degree in the case of coupling, though not of repulsion.

Differential mortality is eliminated by the above method under the same conditions as by the direct method. This may be seen at once from the fact that the coefficient of association is unaltered when (aB) and (ab) are diminished in the same ratio.

It may be remarked that where there is incomplete coupling or repulsion in unequal degrees (in other words finite but unequal cross-over values) in both sexes, the above method of evaluating t gives $t = pq$, where p and q are the cross-over values in the two sexes.

To return to Punnett's sweet pea experiment quoted above: from formula (7) the probable error of P is

$$.477 \sqrt{\frac{2.7743 \times .2257}{2.5486 \times .6952}}, \text{ or } .00284.$$

Hence the cross-over value is $12.00 \pm .28 \%$.

From formula (9) the approximate probable error of X is

$$.6745 \times 8.33 \sqrt{\frac{7.33 + 1.17}{6952}}, \text{ or } .197.$$

Hence

$$x = 7.333 \pm .197.$$

A table of values of $\frac{2}{\sqrt{\pi}} \int_0^x e^{-t^2} dt$ shows that 7 is quite a probable value of x , whilst the chances against an error as large as that involved by $x = 8$ are about 50 to 1.

It would be most desirable to examine all extant linkage data on these lines, and determine whether integral values of x and y , if possible of the form $2^n - 1$, lay within the zone of probable error in about half the cases. Were this found to be the case it would be a strong argument in favour of the reduplication theory, if not it would tend to disprove that theory, at least in its present form.

My thanks are due to Professor Edgeworth, F.B.A., for valuable advice and criticism.

SUMMARY.

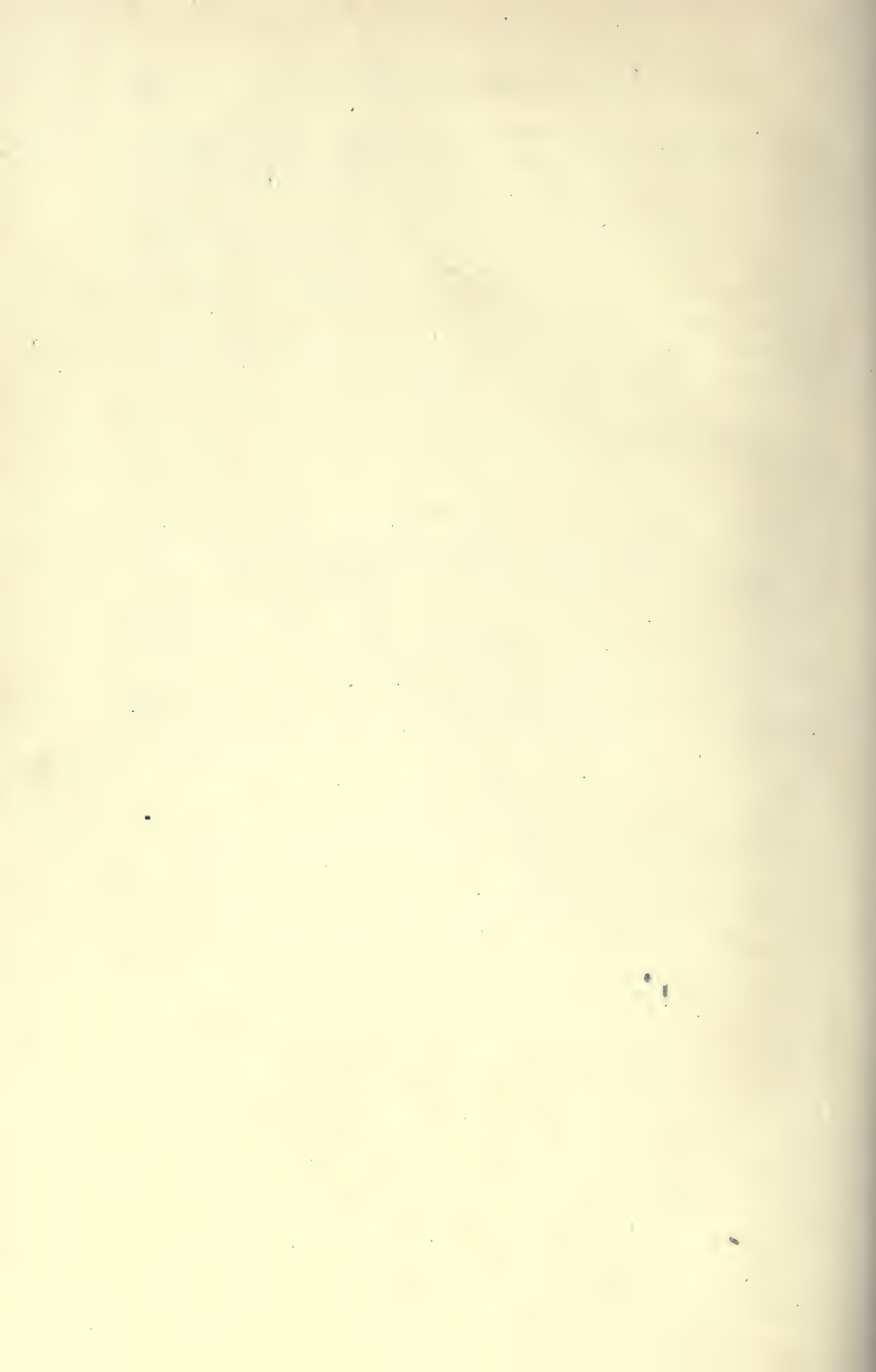
1. Formulae are given for the probable errors of linkage and reduplication values calculated from the offspring of crosses of types $AB.ab \times abab$, $Ab.aB \times abab$, $AB.ab \times AB.ab$, and $Ab.aB \times Ab.aB$.

2. A method is given by which the best possible linkage values may be calculated from the offspring of the latter two crosses, i.e. from F_2 .

3. If this method is employed, F_2 is almost as accurate a means of measuring linkage as are the offspring from $F_1 \times$ double recessive; it is also slightly more sensitive as a means for the detection of linkage.

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THE COMBINATION OF LINKAGE VALUES, AND THE CALCULATION OF DISTANCES BETWEEN THE LOCI OF LINKED FACTORS.

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(With One Text-figure.)

ON the theory that the degree of linkage between two factors depends on the distance apart of their loci in a chromosome, Morgan and his fellow-workers have taken the distance between two loci as proportional to the cross-over value¹ of the factors located in them. This theory gives consistent results when the cross-over values are small, but, as recognised by Sturtevant, and by Morgan and Bridges(1), is not accurate for larger values. On the reduplication theory Trow(2) has given a formula for the combination of linkage values which is shown below to be inaccurate when the linkage is not close. In the present paper a more accurate theory of the relations *inter se* of the cross-over values, and of their connexion with the distances apart of the loci of factors in a chromosome, is developed. Some such theory is especially necessary when dealing with a group of factors containing few members not very closely linked.

Suppose A, B, C to be three factors whose loci lie in that order in the same chromosome. Let m be the cross-over value for A and B , n that for A and C . If the chromosomes were perfectly flexible, so that the fact of their having crossed between A and B did not diminish the probability of their crossing again between B and C , we should expect a triply heterozygous organism to produce gametes in the fol-

¹ If zygotes of composition $AB.ab$ and $Ab.aB$ give gametic series

$(1-m)AB : mAb : maB : (1-m)ab$ and $mAB : (1-m)Ab : (1-m)aB : mab$ respectively, then m is said to be the cross-over value for the factors A and B .

lowing proportions if it were of composition $ABC.abc$, and similarly for other compositions:

No cross-over	$(ABC \text{ and } abc).$	$(1-m)(1-n).$
Cross-over between loci of A and B only ..	$(aBC \text{ and } Abc).$	$m(1-n).$
" " " B and C only	$(ABc \text{ and } abC).$	$(1-m)n.$
" " " A and B and of B and C	$(AbC \text{ and } aBc).$	$mn.$

Actually the last class has been shown to be in defect in many cases. This has been thought to be due to the loops formed by the chromosomes during synapsis having a modal length(3). If this were so, we should expect to find an excessive number of double cross-overs when the distance between the loci of A and C was equal to twice the modal distance between points of crossing over. This phenomenon has however not been recorded. The shortage of double cross-overs can equally well be explained by the mere rigidity of the chromosomes, which makes sharp bending difficult. In the sex chromosome of *Drosophila* the ratio¹ of observed to calculated numbers of double cross-overs is .58:1 for eosin (white), vermilion, and sable (4) (where $m+n = .406$), and .21:1 for vermilion, sable, and bar (3) (where $m+n = .239$).

If the calculated number mn of double cross-overs occurred, the cross-over value for A and C would be equal to the total number of single cross-overs, i.e. to $m(1-n) + (1-m)n$, or $m+n-2mn$.

If double cross-overs were impossible, but the full numbers of single cross-overs occurred, as would happen if the chromosomes were straight rigid rods, the cross-over value for A and C would obviously be $m+n$ (Morgan and Bridges' formula).

Finally if double cross-overs were impossible, and in every case where one should have occurred according to the calculation above, a single cross-over took its place, the cross-over value for A and C would be $m+n-2mn+mn$, or $m+n-mn$. This case might be approximately realised if the chromosome could not form loops shorter than some definite length.

Hence the cross-over values for A and C should be approximately $m+n$ when m and n are small, $m+n-2mn$ when their sum is large, and $m+n-mn$ for intermediate values.

Table I contains the observed values(5) for all triads of factors in the sex chromosome of *Drosophila* for which each of the cross-over values exceeds .1 (10 %). The first column gives the three factors concerned in each case; the second and third columns give the cross-

¹ Called by Muller the "coincidence."

over values for the first and second, and second and third factors respectively, *i.e.* m and n . The fourth, fifth, and sixth columns give the results of the three provisional summation formulae obtained above; the seventh gives the observed crossover value for the first

TABLE I.

1 Factors	2 m	3 n	4 $m+n$	5 $m+n-mn$	6 $m+n-2mn$	7 Observed	8 Class
White sable lethal <i>sc</i> ...	412	236	648	551	454	460	γ
Yellow vermillion rudimentary ...	345	241	586	503	429	439	γ
" " bar ...	345	239	584	502	419	479	γ
White depressed bar ...	203	380	583	506	429	436	γ
" sable forked ...	412	160	572	506	410	457	γ
Yellow sable rudimentary ...	429	143	572	511	449	429	β
" bar ...	429	138	567	508	449	479	γ
White vermillion fused ...	305	258	563	484	406	433	γ
Bifid vermillion forked ...	311	245	556	480	401	425	γ
White sable rudimentary ...	412	113	555	496	437	424	β
Bifid vermillion rudimentary ...	311	241	552	477	402	427	γ
White vermillion forked ...	305	245	550	475	401	457	γ
" sable bar ...	412	138	550	493	436	436	$\gamma\beta$
Yellow miniature bar ...	343	205	548	478	407	479	β
White vermillion rudimentary ...	305	241	546	472	399	424	γ
" " bar ...	305	239	544	471	398	436	γ
" miniature bar ...	332	205	537	469	401	436	γ
Yellow miniature rudimentary ...	343	179	522	471	419	429	γ
White miniature rudimentary ...	332	179	511	452	392	424	γ
" reduplicated bar ...	289	206	495	435	375	436	β
" furrowed forked ...	303	191	494	436	378	457	γ
Bifid miniature rudimentary ...	306	179	485	430	375	427	γ
White furrowed bar ...	303	179	482	428	374	436	β
Yellow vermillion sable ...	345	101	446	411	376	429	β
Facet vermillion sable ...	326	101	427	394	361	430	$\alpha\beta$
Depressed vermillion bar ...	170	239	409	368	328	380	β
White vermillion sable ...	305	101	406	375	344	412	α
Shifted vermillion bar ...	155	239	394	357	320	314	β
White depressed vermillion ...	203	170	373	338	304	305	γ
Yellow club vermillion ...	177	188	365	332	298	345	β
White lethal <i>sb</i> miniature ...	156	199	355	325	295	332	β
White club vermillion ...	143	188	331	304	277	305	β
" lemon vermillion ...	145	120	265	248	230	305	α
Vermillion sable forked ...	101	160	261	245	229	245	γ
" " rudimentary ...	101	143	244	230	215	241	β
" " bar ...	101	138	239	225	211	239	$\alpha\gamma$

and third factors. In the eighth column these observed values are classified as follows:

Greater than $m+n$	α
Between $m+n$ and $m+n-mn$	β
" $m+n-mn$ and $m+n-2mn$	γ
Less than $m+n-2mn$	ϵ

Those exactly equal to $m+n$ are classified as $\alpha\beta$, and so on. The data are placed in the order of the magnitudes of $m+n$. Where any

of the three observed values is based on a count of less than 500 individuals (in which case the probable error of the cross-over value may exceed 1.5 %, as pointed out by the author(6) elsewhere) a query is placed in the last column.

It will be seen that the observed values, when $m+n$ exceeds .5, lie almost wholly between $m+n-mn$ and $m+n-2mn$, as demanded by the theory above. The three discordant values out of 19 are no more than would be expected in view of the probable errors of the observations due both to small numbers and differential mortality. When $m+n$ is less than .5 the results are somewhat more irregular, as the calculated values from the three formulae are not very different, but the majority of observations lie between $m+n$ and $m+n-mn$, as demanded by the theory.

This table also enables us to test the formulae given by Trow(2), based on the reduplication theory. If reduplication takes place so that A and B when coupled give the gametic series

$$qAB:1Ab:1aB:qab \left(\text{cross-over value } m = \frac{1}{q+1} \right),$$

whilst B and C give the series

$$rBC:1Bc:1bC:rbC \left(\text{cross-over value } n = \frac{1}{r+1} \right),$$

then A and C should give the series

$$(qr+1)BC:(q+r)Bc:(q+r)bC:(qr+1)bc$$

$$\left(\text{cross-over value} = \frac{q+r}{qr+q+r+1} \right).$$

This latter value $= \frac{1}{q+1} + \frac{1}{r+1} - \frac{2}{(q+1)(r+1)} = m+n-2mn$. Hence on this hypothesis the observed cross-over values for A and C should cluster round $m+n-2mn$, and approximately equal numbers should be greater or less than it. In other words, as many values should fall in class δ as in classes α , β , and γ together.

The expectation is therefore 18(δ), 18(α , β , and γ); the actual numbers are 3.5(δ), 32.5(α , β , and γ), reckoning the single value $\gamma\delta$ as half in each class. Hence the above form of Trow's theory is untenable.

On a more complicated form of the same theory, which Sturtevant(7) has shown to be impossible on other grounds, A and C when coupled

alone give a primary series $sAC:1Ac:1aC:sac$, and in zygotes of composition $ABC:abc$, a series

$$(qrs + s)AC:(q + r)Ac:(q + r)aC:(qrs + s)ac$$

$$\left(\text{cross-over value} = \frac{q + r}{qrs + q + r + s} \right)$$

As this value is less than that of $m + n - 2mn$, it is still more clearly impossible.

The supporters of the reduplication theory must therefore explain the deficiency of the double cross-over classes of gamete (which from a zygote of composition $ABC:abc$ are AbC and aBc). On the chromosome theory this is due to the rigidity of the chromosomes, and until an equally plausible explanation on the reduplication theory is given, the chromosome theory must be considered the more probable of the two, so far as the class of evidence dealt with in this paper is concerned.

It has been shown above that if A , B , and C are three factors whose loci lie in that order in the same chromosome, and if m and n are the cross-over values for A , B , and B , C respectively, then the value for A and C is $m + n - pmn$, where p is a number between 0 and 2, increasing on the whole with $m + n$, and having the value 1 when $m + n = \text{about } .5$. The distances between loci may now be calculated as follows:

Let x be the distance between the loci of two factors, y their cross-over value, and let the unit of distance be chosen so that when y is sufficiently small x becomes equal to y . This assumption is legitimate if we suppose that crossing over is as likely to occur (other things being equal) at one point in the chromosome as another, *i.e.* that the chromosome is equally flexible and breakable at all points. The unit of distance is thus 100 times Morgan's unit.

If now we write $y = f(x)$, the form of this function being indeterminate,

$$\therefore f(x+h) = f(x) + f(h) - pf(x)f(h), \text{ where } h \text{ is any increment of } x.$$

$$\therefore \frac{f(x+h) - f(x)}{h} = \frac{f(h) - pf(x)f(h)}{h}$$

Now as h is decreased towards 0, $\frac{f(h)}{h}$ tends to the limit 1.

$$\begin{aligned} \therefore \frac{dy}{dx} &= \text{Lt}_{h \rightarrow 0} \frac{f(x+h) - f(x)}{h} \\ &= \text{Lt}_{h \rightarrow 0} \frac{f(h) - pf(x)f(h)}{h} \\ &= 1 - pf(x), \text{ where } p \text{ has the value assumed when } m = y, n = 0, \\ &= 1 - py. \end{aligned}$$

Therefore

$$x = \int_0^y \frac{dt}{1-pt}, \text{ since } x \text{ and } y \text{ vanish together, and } py < 1.$$

Hence if p were constant we should have

$$x = \frac{-1}{p} \log_e (1 - py), \text{ or } y = \frac{1 - e^{-px}}{p} \dots\dots\dots (1).$$

Since however p varies between 0 and 2, the values of x must lie between y , and $\frac{-1}{2} \log_e (1 - 2y)$, those of y between x and $\frac{1 - e^{-2x}}{2}$; the equation

$$y = x \dots\dots\dots (2)$$

being nearly accurate for small values of x and y , the equation

$$y = \frac{1 - e^{-2x}}{2}, \text{ or } x = \frac{-1}{2} \log_e (1 - 2y) \dots\dots\dots (3)$$

for large values of x and y , as is obvious, since for large values of x , y approaches the value .5 asymptotically. The equation (2) corresponds to Morgan's summation formula $m + n$, the equation (3) to Trow's formula $m + n - 2mn$.

The equation (3) may be deduced more directly as follows for a perfectly flexible chromosome:

Let a length x of the chromosome be considered as divided into a very large number N of small equal portions. Then the chance of a cross-over in each of these is approximately $\frac{x}{N}$. Hence the chance of a cross-over in t of these segments and no more is

$$\frac{N!}{t!(N-t)!} \left(\frac{x}{N}\right)^t \left(1 - \frac{x}{N}\right)^{N-t}.$$

When N becomes infinite the limiting value of this expression, *i.e.* the probability of exactly t and no more cross-overs in a length x , is

$$c_t = \frac{x^t e^{-x}}{t!} \dots\dots\dots (4).$$

Hence the value of y for a given value of x is the sum of the probabilities of all odd numbers of cross-overs.

$$\begin{aligned} \therefore y &= c_1 + c_3 + c_5 + c_7 + \dots\dots \\ &= e^{-x} \left(\frac{x}{1!} + \frac{x^3}{3!} + \frac{x^5}{5!} + \frac{x^7}{7!} + \dots\dots \right) \\ &= e^{-x} \sinh x \\ &= \frac{1 - e^{-2x}}{2} \dots\dots\dots (3). \end{aligned}$$

In practice, however, owing to the rigidity of the chromosome, the value of c_1 thus calculated is too small, and those of c_2, c_3 , etc., too large. They are however more accurate for great lengths, where the rigidity of the chromosomes affects the results to a less extent.

It is suggested that the unit of distance in a chromosome as defined above be termed a "morgan," on the analogy of the ohm, volt, etc. Morgan's unit of distance is therefore a centimorgan.

To obtain a more accurate relation between x and y we may plot the curves representing equations (2) and (3), and then obtain empirically a curve lying between the two which fits the observed results as closely as possible. This has been done in the figure, where line (a) represents equation (2), curve (b) equation (3), and curve (c)

$$x = 7y - \frac{3}{2} \log_e (1 - 2y) \dots\dots\dots (5)$$

Equation (5) is merely chosen to give as good a fit as possible and has probably no theoretical significance. The points representing observations are plotted as follows:

The values of y in columns 2 and 3 of Table I are taken, and the corresponding distances in morgans (values of x) read off from curve (c) or Table II, which is calculated from equation (5). These latter are added together, and a point plotted with their sum as abscissa and the observed cross-over value from column 7 of Table I as ordinate. For example the first row of Table I gives the following result:

The cross-over values .412 and .236 correspond, according to the curve (c), or better, by interpolation from Table II, to distances of .549 and .261 morgans respectively. The sum of these distances is .810, and the observed cross-over value from column 7 is .460. The point farthest to the right is accordingly plotted with abscissa .810 and ordinate .460. Curve (c) gives the value .479 for y , and the error of y is accordingly .019, or 1.9 %.

It will be seen that 18 of the observations lie above the curve (c), 18 below, and that in only 4 cases, 3 of which are among the results queried in Table I, does the error of y exceed .04 or 4 %. The probable error of the cross-over values, as calculated from the curve, is 1.8 %, or, omitting the queried results, 1.6 %. This result is not large considering the probable errors of the values of y for the points plotted, which range from 3.1 % downwards.

The curve gives satisfactory results for smaller cross-over values, but these are not plotted, as they do not allow of much discrimination be-

tween the three equations. If the points had been plotted from either line (a) or curve (b), $3\frac{1}{2}$ would have lain on one side, $32\frac{1}{2}$ on the other, as may be seen from Table I.

Hence the curve (c) may be taken as a fairly accurate guide to the combination of linkage values, and this remains equally true whether the chromosome theory is adopted or not. For this reason a series of values of $100x$ and $100y$ (*i.e.* distances in centimorgans and cross-over values as percentages) calculated from equation (5) are given in Table II. As more results accumulate it should be possible to correct these values, which are rather uncertain for large values of x and y .

TABLE II.

100 y (Cross-over value as percentage)	0.0	5.0	8.0	10.0	11.0	12.0	13.0
100 x (Distance in centimorgans) ...	0.0	5.1	8.2	10.3	11.4	12.5	13.6
100 y ... 14.0	15.0	16.0	17.0	18.0	19.0	20.0	21.0
100 x ... 14.7	15.9	17.0	18.1	19.3	20.5	21.7	22.9
100 y ... 25.0	26.0	27.0	28.0	29.0	30.0	31.0	32.0
100 x ... 27.9	29.2	30.5	31.9	33.3	34.7	36.2	37.7
100 y ... 36.0	37.0	38.0	39.0	40.0	41.0	42.0	43.0
100 x ... 44.3	46.1	48.0	50.0	52.2	54.4	56.9	59.6
100 y ... 47.0	48.0	49.0	49.5	49.7	49.8	49.9	50.0
100 x ... 75.1	81.9	93.0	99.2	109.4	117.7	128.1	∞

As an example of the use of this table the following problem may be taken:

"The factors A and B give a cross-over value of 38.5% , the factors B and C a value of 22.7% . What is the value for A and C ?"

From the table we find by interpolation that the distance AB is 49.0 centimorgans, the distance BC 24.9 . Hence the distance

$$AC = AB \pm BC = 73.9 \text{ or } 24.1 \text{ centimorgans.}$$

The cross-over value is therefore 46.8% or 22.0% , according as C lies outside AB or between A and B . Morgan's formula would have given 61.2% (an impossible value), or 15.8% ; Trow's formula 43.7% , or 28.9% (by solving the equation $m + .227 - 2m \times .227 = .385$). On the reduplication theory the result from $AB + BC$ corresponds to the view that the reduplication between A and C is "secondary" to those between A, B and B, C ; the result from $AB - BC$ to the view that the reduplication between A and B is secondary to those between A, C and C, B .

It should be remarked that the existence of a quantity x which has the property demonstrated above is not a conclusive proof of the chromosome theory, and indeed such a quantity may occur in certain forms (*e.g.* Trow's) of the reduplication theory. However the fact that the

values of x correspond to those demanded for the distance on the hypothesis that the factors are located in a semi-rigid chromosome is a strong point in favour of that hypothesis.

We have now the data for a fairly accurate estimate of the total length of the known portion of a chromosome, *e.g.* the sex chromosome in *Drosophila*. Taking some of the best authenticated measurements we have:

Factors	100 x (Cross-over value in per cent.)	100 x (from Table II)
Yellow-White	1.1	1.1
White-Vermilion ...	30.5	35.4
Vermilion-Bar	23.9	26.5
Bar-Lethal <i>sc</i>	8.3	8.5
Totals	63.8	71.5

This gives a total length of 71.5 centimorgans against Morgan and Bridges' estimate (8) of 66.2. The discrepancy is due to the fact that in some comparatively long segments of the chromosome (*e.g.* between the loci of Sable and Rudimentary, a distance of about 15 centimorgans) no factors have been located, and such distances tend to be underestimated. It may also be due in part to the large probable error involved in using a large number of small distances.

From equation (4) we may calculate the proportion of chromosomes giving t cross-overs in the known region. These values are incorrect, owing to the rigidity of the chromosome, c_1 being too low, the remainder too high. The theoretical values are:

No cross-over in $c_0 = e^{-71.5}$, or 49.1% of the chromosomes

One " in $c_1 = .715e^{-71.5}$, or 34.4% " "

Two cross-overs in $c_2 = \frac{.715^2 e^{-71.5}}{2}$, or 12.6% " "

Three " in $c_3 = \frac{.715^3 e^{-71.5}}{6}$, or 3.0% "

Four " in $c_4 = \frac{.715^4 e^{-71.5}}{24}$, or .36% "

and so on.

The value of c_1 is too low, the others too high. The real value of $c_1 + c_2 + c_3 + \dots$ is the cross-over value of 46.3%, and Morgan (8) gives $c_2 + c_4 + c_6 + \dots$ the number of double cross-overs (including quadruples, etc.), as about 10%, so that c_2 should be about 4.3%. When the relation between x and y is accurately known it will be possible to calculate the values of c_t with accuracy by integration.

It is believed that the above method of estimating distances will prove of considerable value when applied to comparatively long chromosomes in which factors are sparsely located, such as the second and third in *Drosophila*, since there is no reason to suppose that the relation arrived at between distance and cross-over value is peculiar to the sex chromosome in *Drosophila*. The results of investigations on these chromosomes should go far to confirm or refute the theory.

Outside *Drosophila* the best series of results on which to test it are those of Altenburg(9) with the three factors *M*, *S*, and *G* in *Primula sinensis*, quoted by Punnett(10) in a recent paper. Here the cross-over value for *M* and *S* is 11.6%, for *M* and *G* 34.0%, for *S* and *G* 40.6%, each result being based on 3684 individuals. By Table II the distance *SM* is 12.1 centimorgans, *MG* 40.9, and hence *SG* is 55.0 centimorgans (assuming the loci to lie in the order *SMG*). Hence the cross-over value for *S* and *G* should be 40.4%, the observed value being 40.6%, a very nearly perfect fit. The addition formula gives 45.6%, Trow's formula 37.7%. The probable error of the calculated result is .64%, of the observed .55%. Hence the probable value of their difference is .84%, and though the close agreement is accidental, both the alternative formulae are impossible.

In the case of Punnett's(10) results for sweet peas the agreement is also good, but owing to the closeness of the linkage, the three formulae give nearly equal values. There is, however, no reason to suppose that Table II does not represent with fair accuracy the relation between distance and cross-over value in all organisms, though the absolute value in $\mu\mu$ of the unit of distance, or morgan, is presumably different in different cases.

SUMMARY.

By a consideration of the observed gametic ratios of the sex-linked factors in *Drosophila*, the following results, among others, are arrived at:

1. If *A*, *B*, and *C* are three factors lying in a chromosome in that order, and if *m* is the cross-over value for *A* and *B*, *n* that for *B* and *C*, then the value for *A* and *C* lies between $m + n$ and $m + n - 2mn$, being nearer to the former when $m + n$ is small, to the latter when it is large.

2. A relation is arrived at, on the hypothesis that the chromosomes are partially rigid, between cross-over value and distance, which permits of the calculation of one of the cross-over values for three factors from the other two, with a probable error of less than 2%.

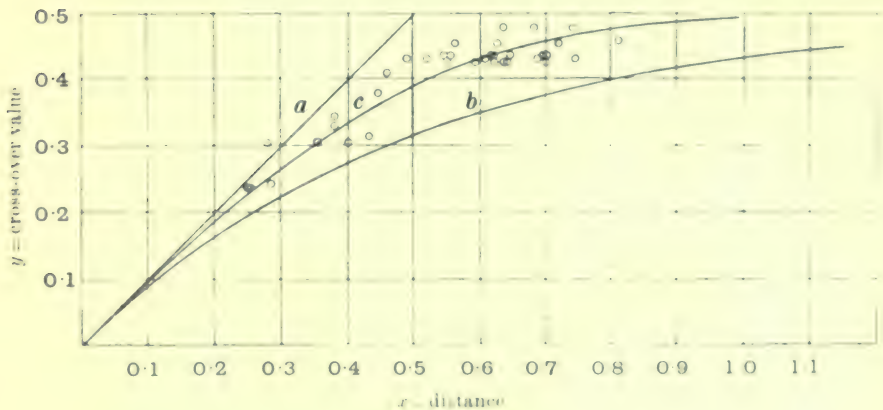
3. This relation may also be used to calculate the total length of a chromosome, and the number of double and triple crossovers to be expected in a large distance.

4. The results from *Drosophila* are incompatible with Trow's form of the reduplication theory, but perhaps not with other possible forms of it.

5. The theory developed above fits all the observed data in plants.

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